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SEXUAL VERSATILITY AND EVOLUTIONARY PROCESSES IN FUNGI [1]

JOHN R. RAPER

Sex, quite aside from its recreational possibilities, has profound and far-reaching biological significance, and the fungi, of all organisms the most diversified as regards sexual manifestations, afford the ideal materials for the dissection of the significance of sexual processes. Evolution, the product of variation, selection, and adaptation, is closely geared to sexual union and the consequent reassortment of genetic characters. By the same token, the sexual characteristics of a given organism must in large measure be both determining for and determined by the evolutionary history of the species. If this relationship is admissible in particular, its application in general leads to a basic riddle: is sexual versatility in fungi the cause or the result of evolutionary diversity? A satisfactory resolution of this enigma can perhaps best await the precedent established in an eventual judgment to be handed down in the celebrated case of *egg vs. hen*.

The diversity of sexual processes in fungi has generated a corresponding diversity of interpretation. Learned disquisitions by Kniep (**57**), Link (**64**), Hartmann (**48**), Whitehouse (**103**), Burnett (**22**), Ahmad (**3**), Olive (**70**), and others (**82**) have sought to bring a measure of order out of the perplexing array of sexual phenomena with results that reflect a wholesome, albeit sometimes not too helpful, spirit of independence and intellectual integrity among mycological sexolo-

[1] Presidential address, presented at the Bloomington, Indiana, Meeting of the Mycological Society of America, August 28, 1958.

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gists, whom, for the next few minutes, let us call mycosynologists. This is an elision of mycosyngamologist, which becomes a bit cumbersome; but please, recognize that the *syn* in mycosynologist is spelled with a *y*. It is difficult to restrain a wish to return to the halycon days just following Blakeslee's 1904 discovery (**11**) of mycelial interaction in *Rhizopus*. It was a simple world with simple alternatives: a fungal species was either homothallic or heterothallic, and since there were only two interacting classes in the heterothallic mucors (**12**), it was commonly accepted that the differentiation was sexual. In this world, the mycosynologist could enjoy a comfortable sense of security in a well ordered field of endeavor. This security was shortly made somewhat uneasy, hence the more cherished, by Edgerton's report (**37**) of cross-fertility between self-fertile strains of *Glomerella;* but, why bother? *Glomerella* would jolly well have to declare itself in less ambiguous terms. Early in the third decade of the century, however, the simplicity and orderliness of the mycosynologist's world began to crumble in earnest. Kniep (**53, 54**), in frequent and amazingly perceptive papers, was busily sketching the broad outlines of a pattern of sexuality in the Basidiomycetes that was explicable in terms of sexual differentiation only through an outlandish distortion of age-old concepts about sex. When viewed from this bias, the preliminary definition of tetrapolar sexuality, which stated that each individual mushroom produced progeny of four different sexes, was disturbing enough. Subsequent emendations to the effect that different individual mushrooms produced progeny having different sets of four sexes (**55**) and that the sexual factors themselves were subject to frequent mutations (**56, 58**) brought tetrapolarity to the brink of heresy. Meanwhile, in 1923 and 1924, the discovery by Vandendries (**98**), Newton (**18**), and Brunswik (**17**) that some species of mushrooms could make do with only two sexes per individual must have afforded a bit of welcome relief. This respite, however, was short-lived and more than offset by the discovery by Couch in 1926 of yet another pattern of heterothallism (**25**), this time in the aquatic phycomycete *Dictyuchus*. Here there was good morphological differentiation between male and female in the mating of two individuals, but there was a fascinating uncertainty about who was which in more extensive confrontations, because certain individual strains had the happy facility of reacting equally well as male with one mate and as female with the next. The lines of perplexity were fully drawn with the entry of an ascomycete into the field, when in 1927 Shear and Dodge (**93**) found that certain monilias in the proper company became perfectly respectable neurosporas. Later work of Dodge (**30, 31**), Ames (**4**), and others (**5, 33, 34, 35**) revealed

the sexual amenities here again to be novel: each of the two interacting classes produced differentiated and functional organs of both sexual signs, male and female, but sexual fertility could only result from the interstrain transfer of nuclei.

During the three succeeding decades there has been amassed a large literature that relates to the sexual idiosyncrasies of the fungi. Sexuality has been described in a large number of new species, and some very unusual manifestations of sex have been unearthed. It is helpful to recognize, though, that practically all of this recent work comprises an extension of the few basic patterns of sexuality listed above in their approximate historical context. We have reinterpreted them, shown some of their operational anomalies, built genetic underpinnings under some of them, delved more deeply into their physiological aspects, and in rare bursts of courage and braggadocio have even tried to rationalize their origins (**22, 70, 81, 82, 104**). And we aren't through yet; we still have a good supply of unused adjectives and prefixes: to partial heterothallism (**67**), relative heterothallism (**74**), balanced heterothallism (**70**), and unbalanced heterothallism (**70**), we shall in time add pseudo-, crypto-, ambi-, quasi-, brachy-, euheterothallism, an academic equivalent for "My, that's a real baby you have there!," and polyheterothallism, a situation best described by E. B. Browning's "How do I love thee?//Let me count the ways" (**16**).

So far, this concerns only the overt sexual characteristics and activities of the fungi. In addition, there are two cryptic processes that are of common occurrence in the fungi and that possibly have sexual significance equal to that of the routine sexual processes. (1) Heterokaryosis associates genetically dissimilar nuclei in a vegetative system to which accrue the essential benefits of sexual union without its entangling permanence. Heterokaryosis is apparently a fungal monopoly (**8, 13, 14, 32, 45, 46, 74, 84, 89, 91, 92**). (2) Somatic recombination or parasexuality (**74, 75**) reassorts genetic characters of heterokaryotic components in a vegetative system to yield products that are comparable to the meiotic products of the sexual process. Somatic recombination has been demonstrated in numerous fungal groups (**23, 72, 74, 76, 77, 78, 92, 97**); it is also known to occur in other organisms—Stern's original demonstration of the phenomenon was in *Drosophila* (**96**)—but to date, only in the fungi has it been shown to be incorporated into the genetic continuum of the organism. Heterokaryosis and somatic recombination, singly or together, mimic true sexual processes and may also serve to a surprising degree as biologically effective substitutes for sex, the former on a temporary, the latter on a permanent basis (**84**).

Underlying these overt and cryptic sexual processes is a basic principle of fungal organization; the high degree of vegetative-sexual interconvertibility (**10**, **39**, **52**, **80**). It is not true to say of most fungi that they have no germplasm, because to all intents and purposes they have nothing but germplasm. This characteristic is shared to varying degrees by certain other microorganisms, but particularly in the fungi, any changes that occur in the genetic equipment of vegetative cells have maximal opportunity of being transmitted to future generations.

These, then, are the basic aspects of sexuality in the fungi, and together the various processes impart to the group generally a plasticity unequalled elsewhere. When the distribution of these several processes among the various smaller categories of fungi is considered, it is evident that few, if any, species are devoid of sexuality—when genetic reassortment of characters is taken as the essential criterion of sex. Furthermore, it is probable that a majority of all fungal species combine overt and cryptic sexuality: excluded here would be only the Phycomycetes, in which heterokaryosis as a genetic balancing device (i.e., an approach toward functional diploidy) apparently does not occur, and the Fungi Imperfecti, in which overt sexuality is lacking, quite possibly because it has turned out to be more bother than it is worth.

If our premise of a close necessary correlation between sexual and evolutionary processes is admissible, and in view of this unparalleled versatility in matters sexual, why haven't the fungi amounted to more than they have? As mycologists, we love them, and as rational beings, we appreciate what they do for us, but let's face it: the fungi have not been able to lift themselves out of the class of garbagemen to the entire organic world. Admittedly, they aren't the lowest of the low; their garbage is cleaned up by bacteria. But why, with the fantastic evolutionary potential that they now have and presumably have had for millions, if not billions, of years—why haven't they amounted to something really stupendous? Again, as mycologists who love them, let us face another bitter fact: they seem to lack initiative in the things that really matter, for they appear to be wholly uninspired by the grandiose structural achievements of their neighbors, green plants and animals, the only other phyletic lines that have evolved well-adapted terrestrial and amphibian forms (**89a**). If, on the other hand, their highest aspiration was to be the very best possible garbagemen and their evolutionary potential was spent largely in this achievement, who are we to blame them for having squandered the remainder of that potential in unusual diversions to relieve the tedium of a humdrum existence? I shall not cast the first stone!

Sexual processes to this point have been considered in the light of their efficacy in promoting genetic reassortment. A continuation of this emphasis requires some justification as regards forms that are self-fertile, or homothallic, a condition that predominates in many groups of fungi. The central fact of homothallism, the ability of sister nuclei to fuse, would appear to choose the expedient safety of resistant sexual products at the expense of the long-term benefits of genetic variability. This way lies extinction, and the hard choice between immediate and eventual extinction must have somewhere a practical loophole. Two mitigating factors have been suggested (**70**, **82**) as probably occurring with sufficient frequency to afford effective relief: (1) The ability of sister nuclei to fuse does not exclude the fusion of non-sisters from different clones that may have widely divergent backgrounds. Interclonal crosses, which often highly favor cross- over self-fertilization, underlie genetic analyses in such homothallic species as *Aspergillus nidulans* (**74**), *Sordaria fimicola* (**24**, **41a**, **69**), *Glomerella cingulata* (**101**, **102**), etc., and the phenomenon can no longer be considered a laboratory curiosity. (2) The accumulation of spontaneous mutations during vegetative growth must frequently result in fusion between nuclei of dissimilar genotypes. Following extensive multiplication, particularly in forms in which there is localized differentiation of complementary sexual organs, syngamy involving completely isogenic elements might well even be exceptional. Thanks to these two extenuations, the homothallic plant cannot properly be thought to be suspended in genetic and evolutionary limbo; evolutionary gains may be maddeningly slow, but the risks are kept minimal. By comparison, obligatory cross-mating must speed up the evolutionary process, but the risks are correspondingly increased. The situation has its counterpart at the poker table: why abandon the modest security of a pair of jacks for the impossibly long odds to fill a royal flush? It may be incidental that the simpler, and presumably more primitive, fungi are predominantly homothallic while the more highly specialized groups are predominantly heterothallic with a high degree of outbreeding; on the other hand, this correlation probably reflects an evolutionary progression, in the course of which the several types of heterothallism evolved. The present pattern of distribution of the different types of heterothallism would thus reflect their relative antiquities.

Let us look, then, a bit more closely at the distribution of the different patterns of sexuality and cryptic sexual processes as they may be correlated with the commonly accepted phylogenetic groupings.

A majority of the Phycomycetes are homothallic, but among the

heterothallic minority there is a remarkable variety of differentiative devices. Heterothallism in the simple, holocarpic forms may reflect an underlying genetic differentiation, or it may follow from a sexual ambivalence that permits each individual to make the most of unexpected opportunities. There is indicative evidence of the former in a few isolated cases (**26**), but for the most part, the uncertainties expressed by Sparrow (**95**) and Karling (**51**) more than a decade ago still apply. Analysis in such forms will not be easy, but it should prove most rewarding, and it is badly needed. At the more sophisticated level of *Allomyces,* a typically homothallic form, a close approach to dioecious heterothallism has been made in the laboratory via hybridization and selection by Emerson and co-workers (**40**). New glamour has been added to this derived and functional heterothallism by Machlis's recent discovery, isolation, and characterization of a hormone that is secreted by female gametangia and gametes and that serves as a chemotactic lure for male gametes, a substance most appropriately named "sirenin" (**65, 66**). Homothallism in *Allomyces* possibly represents a balanced polygene system that has been synthesized from an earlier dioecious separation of the two sexes. These forms are exceptional in that they are diploid, and in them homothallism should have certain selective advantages that haploid forms could ill afford.

In the biflagellated water molds, the whole pattern of sexuality appears to be in a most delightful state of flux. Again, most species are homothallic, but there are occasional heterothallic species—or, at least, predominantly self-sterile and cross-fertile strains that obviously prefer a more social, cooperative type of sexuality than afforded by the narcissistic practice of homothallism. If the genus *Achlya* can be regarded as representative, however, heterothallism is practiced here without any deep conviction (**79, 80**). The results of current work of Barksdale (**7**) happily confirm and significantly extend the speaker's earlier findings and serve to exemplify the state of near anarchy rampant in this group; following are a few points of significance: (1) In each of the two heterothallic "species," *A. bisexualis* and *A. ambisexualis,* most if not all isolates are capable of a sexual ambivalence in which maleness and femaleness are determined in each mating by common consent of the mated. (2) Monoecism can be induced in many of these isolates by the simple expedient of changing the temperature. (3) Isolates assigned to each of the heterothallic species vary widely in morphology and severally resemble, often quite closely, various homothallic species. (4) Inter-specific matings of isolates of these so-called heterothallic species give a range of sexual interaction from near mutual indifference to

the production of matured zygotes. (5) Inter-specific and even inter-generic matings between heterothallic and homothallic isolates are frequently interactive and not rarely are interfertile. The demonstrated role of sexual hormones in many of these interactions helps to rationalize the stages of sexual achievement in specific cases; more importantly, the degree of correspondence of sexual hormones among different species must indicate biochemical and phylogenetic relationships (**83, 85**). At the present time, these relationships are uncertain, and available information is sufficient for only the most generalized hypotheses. But beyond a doubt, here is a group of plants in sexual transition, and if the evidence from their hormones can be trusted, all its members, homo- and heterothallic, are equally committed to those ever inscrutable goals to which their present uncertainties will eventually drive them. It's a most intriguing and challenging problem.

In sharp contrast to that in the water molds, sexuality in the Mucorales appears conservative and well-defined. The classical work of Blakeslee (**11, 12**), Burgeff (**20, 21**), and others (**59, 90**) on sexuality in this group has been the subject of no major revision and surprisingly little extension, and heterothallism as practiced in the Mucorales remains the simplest and least variable differentiative device known in the fungi. The precise nature of the differentiation, whether sexual or incompatibility, remains to be determined, but the over-all pattern of stability in sexual characteristics connotes the almost finished product of an evolutionary progression that has run its course. Imperfect hybridization in the mucors remains as a vestige of past explorations and has apparently lost any present biological effectiveness, except possibly in respect to parasitism (**21**).

In the simpler Ascomycetes, we find again a group in which no fixed attitude toward sexuality has been achieved. Life cycles here run the gamut of haploid, haploid-diploid, to diploid, and in many of the subgroups, the pattern of sexuality varies from homothallic, through numerous transitions (**60**), to heterothallic (**105, 106**). Heterothallism here, however, carries no sexual differentiation; determination of mating behavior by incompatibility factors, which can be defined as simply segregating genetic characters that determine mating competence in the absence of morphological differentiation, characterizes the heterothallic yeasts as, with few exceptions, it characterizes all of the cross-reacting higher fungi. Incompatibility in the yeasts, however, appears to lack the stability that it has achieved in higher forms, for mutations of mating factors to new functional states are reported to be commonplace in many yeasts (**1, 2, 61, 62, 63**).

A single exceptional group of Ascomycetes retains an apparent sexual differentiation (**9**). Members of the highly specialized, insect-inhabiting Laboulbeniales, when heterothallic, are clearly dioecious. Caution should be exercised in the evaluation of this differentiation, however, for the same uncertainty noted with the holocarpic Phycomycetes applies here: there is no experimental evidence for any sexual predestination, and the male-female pairs may represent a physiological accommodation that is possible between any two contiguous, developing thalli. That there is no genetic differentiation of sexual strains here appears likely in view of the prevalence of hermaphroditism throughout the Euascomycetes.

In the remainder of the Ascomycetes and in the rusts of the Basidiomycetes, a simple incompatibility mechanism superposed upon hermaphroditism characterizes cross-mating or heterothallic species (**19, 27, 28, 31, 33, 35**), a pattern of sexuality typified by the 8-spored species of *Neurospora*. The few exceptions to this pattern that have been reported as showing sexual differentiation, e.g., *Stromatinia* (**36**) and *Hypomyces* (**38, 47, 50**), could equally well be explained by aberrations that prevent the normal sequence of development of sexual organs in basically hermaphroditic forms. Mutations that prevent the initiation of protoperithecia are known in *Neurospora* and other forms (**6, 41, 107, 108**). Two such blocks affecting different stages of the sexual process would result, in the absence of incompatibility, in the *Stromatinia* pattern; superpose them on an incompatibility differentiation, and the *Hypomyces* pattern would be effectively mimicked if not actually duplicated. A few other Ascomycetes, such as *Glomerella* (**102**) and *Chromocrea* (**68**), appear to be undecided on the matter of sexuality; these forms are of extreme interest in respect to the operational details of sexual evolution and will be considered later.

Heterothallism in the Basidiomycetes with the exception of the rusts is clearly based on a different sort of incompatibility mechanism from that in the Ascomycetes. Numerous alternate states of one or of two series of incompatibility factors stand in marked contrast to the simple pair of factors so commonly found in the Ascomycetes (**82**). Although they possess no sexual organs, the Basidiomycetes have evolved the most outlandish, free-wheeling, sexual promiscuity without a blushing trace of exhibitionism: every vegetative cell is a potential gamete, and in the fusion process, each cell acts both as donor and as recipient of fertilizing nuclei. Ideas of sexual dimorphism, however, die hard, and there is a report by Greis (**43**) of small, donor, male mycelia and large, receptor, female mycelia in *Solenia anomala*. Hartmann, who

had previously found sexuality in the Hymenomycetes to be a bit too relative, enthusiastically accepted this interpretation of sexual dimorphism and has used it extensively in support of his theory of the universality of relative sexuality (**48**, **49**). A simple, unilaterally mating, morphological mutation—like any one of a number that we have in *Schizophyllum* (**88**)—closely linked to one of the incompatibility factors would explain the findings of Greis in a trice. The original Greis dikaryon of *Solenia* is available (CBS) and, with a cross-reacting stock from nature, is subject to direct test. Such a test, may, of course, end up by exactly confirming Greis's results, but I consider it extremely unlikely that *Solenia* is *so* anomalous as all that!

The two cryptic sexual processes, heterokaryosis and somatic recombination, are more widely distributed among fungi than is any one of the specific patterns of overt sexuality. Heterokaryosis is common, if not actually universal, among the Euascomycetes, the Basidiomycetes, and the Fungi Imperfecti. The operational details of heterokaryosis vary widely from group to group, and they are subject to extreme modification by the incompatibility mechanism whenever this is present (**84**). The effects of the incompatibility mechanism on heterokaryosis can be shown by two examples: (1) the contrasted ease and difficulty of heterokaryotization between like and different mating types in *Neurospora* (**8**, **44**, **91**) and (2) the array of strikingly different heterokaryons in a typical tetrapolar Hymenomycete, such as *Schizophyllum*, as the result of specific incompatibility interactions—the dikaryon, the common-A, or "flat," heterokaryon, the common-B, or "barrage," heterokaryon, and probably even a common-A-common-B heterokaryon (**89**).

Somatic recombination in the fungi seems to be a routine corollary of heterokaryosis, with one probable exception: in heterokaryons constituted of incompatible nuclear types. Too little work has been reported to validate this postulate properly, but there are a few bits of indicative evidence in its support: (1) the failure to demonstrate parasexual recombination in heterokaryons of like mating type in *Neurospora* (**42**; N. H. Giles and Mary Case, T. H. Pittinger, personal communications), (2) the general lack of evidence of somatic recombination in such sexually incompatible heterokaryons in any group, and (3) the widely demonstrated effectiveness of incompatibility mechanisms in the prevention of fusion of incompatible nuclei. In other and fewer words, incompatibility probably applies the same ultimate stricture to legally sanctioned unions and to illicit affairs. Otherwise, somatic recombination has been found wherever it has been intensively sought: (1) in heterokaryons of forms lacking any overt sexuality, e.g., *Penicillium chryosogenum* (**77**), *Asper-*

gillus niger (**76**), and *Fusarium oxysporum* (**23**), (2) in homothallic forms, e.g., *Aspergillus nidulans* (**74**), and (3) in the fully compatible heterokaryon, the dikaryon, of tetrapolar Hymenomycetes (**29, 72, 78**) and of certain rusts (**15, 97, 99, 100**, and A. H. Ellingboe, personal communication. It should be noted that with one exception (**97**) the term somatic recombination has not been specifically applied to the origin of new races following mixed infections of dikaryons in rusts, although the authors well recognized the probability of an underlying parasexual process). In the Hymenomycetes, recent work of Crowe (**29**) has indicated somatic recombination—the now certain basis of the long disputed illegitimate Buller Phenomenon—to be of surprisingly common occurrence in healthy, and, presumably, happy dikaryons.

One important point about all of this is the possible indication that it provides regarding the relative age of the various sexual phenomena and of their interrelationships. The speaker reads this record, whether correctly or not remains to be seen, as signifying great antiquity for heterokaryosis and somatic recombination and somewhat lesser, but still impressive, antiquity for the two distinctive types of incompatibility control found in the Ascomycetes and the Basidiomycetes. The correlation of the two incompatibility mechanisms with numerous other characters, such as the distinctive morphological features of the two groups and biochemical differences, e.g., the common biotin deficiency in the one group and of thiamine in the other, leads to the conclusion that these two distinct differentiative devices are as ancient as are the Ascomycetes and the Basidiomycetes themselves. The dikaryon, with its paralleled associated structures in the two groups, croziers *vs.* clamp connections and asci *vs.* basidia, constitutes a highly specialized system that occurs in no other group of organisms and connotes a common origin for these two major groups of fungi; this possibility at least suggests a common origin for the different incompatibility devices. The further consideration that incompatibility mechanisms occur only in fungi and in higher plants (Pteropsida of the Trachaeophyta) adds some weight to the probable extreme rarity of the genesis of incompatibility as a device favoring outbreeding.

Differentiative devices other than incompatibility seem by comparison to be only a hop, skip, and jump from homothallism, if indeed they have managed the whole of this strenuous sequence to date. A few mutative deficiencies, a bit of polyploidy here and there, and occasional phenotypic differentiation could explain the lot of them. Whether or not they can rightly be dismissed so summarily as all this is, of course, a moot point;

certainly, on the available evidence, I should not care to attempt a strong defense of this thesis.

There is less evidence of wholesale evolutionary change of sexual processes in groups characterized by incompatibility, but species that appear to be in transition are by no means rare. Three examples will suffice: (1) In *Glomerella,* frequent mutations at one locus and occasional mutations at another provide two sets of alternate alleles the assortment of which yields a number of cross-reacting strains that have many of the attributes of mating types in obligate heterothallic species (**102**). Numerous mutative blocks in the sexual progression further favor cross- over self-fertilization (**101**). (2) In *Chromocrea,* there is a simpler but equally puzzling situation (**68**). Sexual interaction appears to occur only between the wild type and a spontaneous mutation that invariably occurs in the wild type. That all asci contain wild and mutant segregants in a ratio of 1:1 indicates that the cross reaction is obligatory. In culture, the mutant is stable and self-sterile but cross-fertile with the wild type; if the wild type were also stable, this system would be indistinguishable from common ascomycetous incompatibility. (3) In the Basidiomycetes, there are also occasional exceptions to the common patterns of sexual behavior. The life cycle of the common honey mushroom, *Armillaria mellea,* has so far eluded all attempts towards its definition. The species appears, from preliminary studies of Snider (**94**), to be neither homothallic, bipolar, nor tetrapolar; true diploidy would seem at present as promising an hypothesis as any for further study. The details of the life cycle of the common field mushroom, *Psalliota campestris,* are little if any better understood. These forms appear to be transitional, but it is difficult to decide in what direction they are progressing; whether they aspire to challenging new expressions of heterothallism or are simply relapsing toward comfortable homothallism.

The query "What does *Glomerella, Chromocrea,* or *Armillaria* mean in reference to incompatibility control in the Ascomycetes or Basidiomycetes?" prompts, at our present stage of understanding, the far more pertinent question "What, pray tell, is incompatibility control in any fungus?" The literature, so far as I can make out, is exactly mute on the subject of *how* incompatibility prevents the sexual interaction of incompatible strains and the fusion of like nuclei in any species. Speculation on the physiological details of incompatibility, whether complementary or oppositional, etc., with the information now available is a bootless business—not enough is known about it to provide an ade-

quate platform from which to launch an honest conjecture. Even the point in the sexual progression at which the incompatibility block is effected is only known in a few cases.

In work now in progress, Bistis (personal communication) has shown that incompatibility effects blocks at two distinct stages in the sexual process in *Ascobolus stercorarius:* (1) prior to ascogonial induction by the sexually activated oidium and (2) following fusion of the trichogyne with the oidium. Ascogonial induction does not occur in the presence of an untreated oidium of the same mating type, nor does it occur following sexual activation of such an oidium by a mycelium of the opposite mating type. Such activated oidia, however, will direct the growth of trichogynes of previously induced ascogonia of the same mating type and will fuse with them in what appears to be normal plasmogamy. Nothing, however, comes of this union; development of an apothecium is arrested at an early stage, and no asci are formed. The first of the inter-strain reactions seen in *Ascobolus* can have no counterpart in most other incompatibility-differentiated species, e.g., *Neurospora,* where abundant proptoperithecia are produced by strains of both mating types in isolation. Whether the incompatibility block in *Neurospora* is comparable to the second block in *Ascobolus* is of the utmost relevance in unraveling the sexuality of the larger group to which both of these forms belong.

In the Basidiomycetes, as judged from the literature generally and our own work on *Schizophyllum* in particular, discrimination due to incompatibility is an intracellular process about which only a few inferences can be drawn at present. Hyphal fusions apparently occur between juxtaposed mycelia of the same species regardless of their incompatibility relationships. Such fusions establish heterokaryotic cells, and the course of future developments is determined after this initial nuclear association.

A great deal of information at the level of operational detail is badly needed for both of these incompatibility systems before any real assessment can be made of their probable evolutionary relationship. One point of differentiation between the two systems is so striking, however, as to prompt a final speculation: the contrast between paired and multiple allelic incompatibility factors in the two groups. The dikaryon is common to both: as the restricted, parasitic stage of ascigerous hyphae in the Ascomycetes and as an independent generation in the Basidiomycetes. If the two groups originated from a common ancestral type, whatever original alteration caused the divergence of the two dikaryotic

types would have of necessity endowed the independent type with a number of critical features. Not the least of these would be the high selective value, particularly via the Buller Phenomenon, of functionally new incompatibility alleles (**67**, **71**, **73**, **104**). These could have accumulated in various ways: either by mutation, or perhaps more probably by duplication and rearrangement of pre-existing loci. *Schizophyllum* is estimated to have 340 and 64 specific factors in its two respective incompatibility series (**87**), and these provide > 21,000 different specific mating types. Since each factor is now known to be constituted of two subunits, only one of which need be heterozygous for full compatibility (**86**), the entire system could operate on only sixty or so different genetic specificities or alleles—and this number may well be reduced by further resolution of the incompatibility loci.

Three important conclusions can be drawn from the considerations to which attention has been directed in this presentation. (1) As judged solely by sexual characters, the fungi constitute an evolutionary cul-de-sac, a conclusion so well bolstered from every side as to seem trivial. (2) There is abundant evidence that the evolution of sexual processes in the fungi has paralleled the morphological and physiological divergencies that have produced the major groups of fungi, and that a more thorough appreciation of sexuality is essential to a valid interpretation of the course of fungal evolution. (3) The detailed information relating to sexuality now available, however, is still too fragmentary to support anything more tangible than the broadest interpretive synthesis. It is my own belief that sexuality provides one of the most critical keys, if not the most critical, to an eventual rationalization of the diversity of fungi. Besides all of which, the study of sexuality in any group of fungi or in the fungi generally has a challenge and a fascination—a propensity for surprises—all its own.

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fungi, were most stimulating—despite the fact that indigestion was achieved far more readily and more frequently than was agreement.

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CROSS-RESISTANCE PATTERNS OF MUTANTS RESISTANT TO STREPTOMYCES PHAGES[1]

S. GAYLEN BRADLEY

(WITH 2 FIGURES)

INTRODUCTION

Interspecific heterokaryons are formed between strains of *Streptomyces griseus, S. olivaceus, S. cyaneus* and *S. venezuelae* (Bradley and Lederberg, **1956**). Hyphal fusion between two filamentous fungi is indicative of taxonomic affinity. Bacteriophages have also been used successfully to identify bacteria (Felix, **1956**). Conversely host range can be used to show relationships among different bacteriophages (Adams, **1955**). Analysis of cross-resistance patterns of streptomycetes selected for resistance to bacteriophage increases the discrimination of the system. This study compared 12 *Streptomyces* phages and 7 strains of *Streptomyces* by this method.

METHODS

Streptomyces venezuelae strain S13 was kindly supplied by Dr. J. Lederberg, University of Wisconsin. *S. griseus* strain S1945 was kindly supplied by Dr. Elizabeth McCoy, University of Wisconsin. The other strains of *Streptomyces* were isolated from soil samples collected in Madison, Wisconsin, and Evanston, Illinois. These included *S. griseus* strains S86, S104 and S34, *S. olivaceus* strain S11 and *S. cyaneus* strain S45. Stocks were grown at 30° C on a glucose-salts medium composed of glucose, 20 g; yeast extract, 1 g; KNO_3, 2 g; K_2HPO_4, 2 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCO_3$, 0.25 g; agar, 15 g; de-ionized water, 1 liter. The medium was autoclaved at 15 lb pressure for 12 minutes.

Bacteriophages W1 through W5 were isolated from Wisconsin soil samples whereas bacteriophages M1 through M4 were isolated from Minnesota soil samples. The method used to isolate bacteriophage was as follows: Soil was added to a 24 hour peptone-yeast-extract broth culture of *S. griseus* strain S86 or S104. The culture was incubated

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overnight at 30° on a rotary shaker. The broth cultures were clarified by centrifugation and rendered bacteriologically sterile by filtration. The presence of bacteriophage in the enrichment was detected by plating 0.01 ml of the enrichment with sufficient fragmented aerial hyphae of *S. griseus* to yield confluent growth on peptone-yeast-extract agar after 24 hours. Peptone-yeast-extract agar was composed of peptone, 5 g; yeast extract, 3 g; agar, 15 g; de-ionized water, 1 liter. The bacterio-

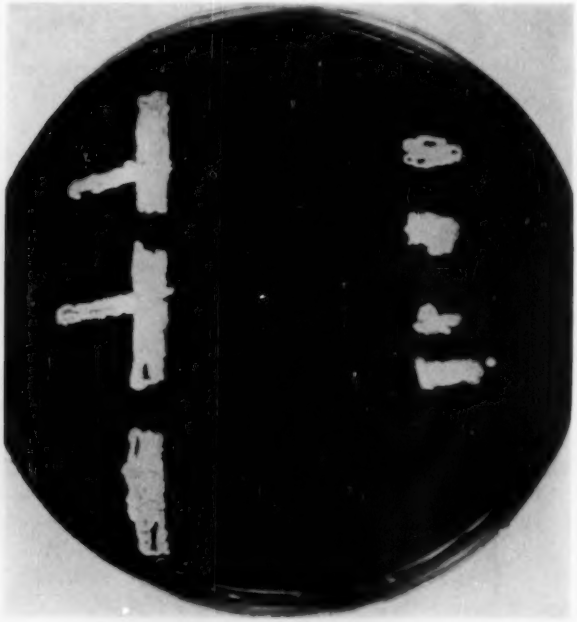

FIG. 1. Reaction of sensitive and immune *Streptomyces* to bacteriophage. Left: cross-streaks of different bacteriophages against an indicator host. Right: cross-streaks of sensitive *Streptomyces* against a bacteriophage.

phage produced discrete areas with no growth; these plaques were picked and replated. After several replatings from a single plaque, a stock virus suspension was prepared. A single plaque was added to a 24 hour culture of the appropriate streptomycete host. After shaking for 24 hours at 30°, the lysate was clarified by centrifugation and preserved with chloroform.

Bacteriophages L1, L2 and L3 were temperate bacteriophages obtained from autolyzing cultures of *S. griseus* strains S1947, S1945 and S104 respectively. Stock virus suspensions were prepared as described above.

Resistant mutants of *Streptomyces* formed discrete colonies after 2 days on peptone-yeast-extract agar seeded with 10^6–10^7 spores and an excess of bacteriophage. In order to obtain lysates with sufficiently

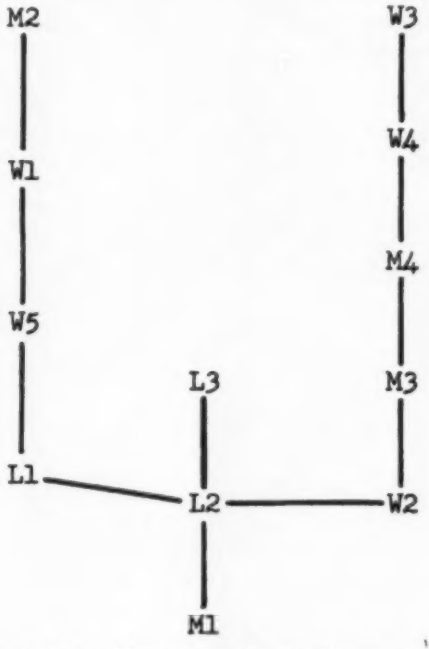

FIG. 2. Relationship among *Streptomyces* phages: adjacent phages are similar; remote phages are dissimilar.

high titers, with respect to a particular host, it was frequently necessary first to grow the bacteriophage on the homologous host. The mutant streptomycetes were purified by serially replating single colonies on glucose-salts agar. Only fully resistant mutants, i.e., those with an efficiency-of-plating less than 10^{-8}, were retained for further study.

Bacteriophage susceptibility was determined by cross-brushing hosts

against a streak of lysate (FIG. 1). All lysates were standardized at 10^9 phage/ml.

RESULTS AND DISCUSSION

Resistance to one bacteriophage frequently conferred resistance to other bacteriophages. For example, *Streptomyces griseus* strain S104 was sensitive to 11 of the 12 phages used; whereas the variant of S104 selected for resistance to W2 was sensitive to only 4 of the 12 phages (TAB. 1). This indicated some relationship among the 7 phages which were avirulent for the W2 resistant mutant of S104. Similarly considering the other data, the phages may be arranged according to their

TABLE 1

BACTERIOPHAGE-RESISTANCE PATTERNS OF MUTANT STREPTOMYCES

Strain*	Phage**											
	W1	W2	W3	W4	W5	M1	M2	M3	M4	L1	L2	L3
S104	+	+	+	+	+	+	−	+	+	+	+	+
S104(W1)	−	+	+	+	−	+	−	+	+	−	±	±
S104(W2)	−	−	+	+	−	−	−	+	+	−	−	−
S104(W3)	+	+	−	±	+	+	−	−	−	+	+	+
S104(W3)	+	+	−	−	+	+	−	−	−	−	−	−
S104(W3)	+	−	−	−	+	−	−	−	−	−	−	−
S104(W3)	+	+	−	+	+	+	−	+	+	+	+	+
S104(W4)	−	−	+	−	−	−	−	+	+	−	−	−
S104(W5)	−	+	+	+	−	−	−	+	+	−	−	−
S104(M1)	−	+	+	+	−	−	−	+	+	−	−	−
S104(M3)	−	−	+	+	−	−	−	−	−	−	−	−
S104(M4)	+	+	−	−	+	+	−	−	−	−	−	−
S104(L1)	−	−	+	±	−	−	−	+	+	−	−	−
S104(L2)	−	−	+	+	−	−	−	−	+	−	−	−
S104(L3)	−	+	+	+	−	−	−	+	+	−	−	−
S86	+	−	+	+	+	+	+	+	+	+	+	−
S86(W1)	−	−	+	+	−	−	−	+	+	−	−	−
S86(W3)	+	+	−	+	+	+	+	+	+	+	+	−
S86(W4)	−	−	−	−	−	−	−	−	−	−	−	−
S86(W5)	−	−	±	+	−	−	−	+	+	−	−	−
S86(M1)	−	−	+	+	−	−	−	+	+	−	−	−
S86(M2)	−	−	±	+	−	−	−	+	+	−	−	−
S86(M3)	+	−	−	−	+	+	+	−	−	+	+	−
S86(M4)	+	−	−	−	+	+	+	−	−	+	+	−
S86(L1)	−	−	+	+	−	−	−	+	+	−	−	−
S86(L2)	−	−	+	+	−	−	−	+	+	−	−	−
S1945	−	+	−	−	−	−	−	−	−	−	−	−
S1945(W2)	−	−	−	−	−	−	−	−	−	−	−	−

* S( ) represents the mutant Streptomyces selected for resistance to bacteriophage ( ).

** +, complete lysis; ±, partial lysis; −, no lysis.

TABLE 1—*Continued*

Strain*	Phage**											
	W1	W2	W3	W4	W5	M1	M2	M3	M4	L1	L2	L3
S11	−	+	±	+	±	±	−	−	−	±	±	+
S11(W2)	−	−	+	+	+	−	−	−	−	−	−	−
S11(W3)	±	±	−	±	+	+	−	−	−	+	+	+
S11(W4)	−	+	−	−	+	+	−	−	−	+	+	+
S11(W5)	−	−	+	+	−	−	−	−	−	−	−	−
S11(M1)	−	+	+	+	−	−	−	−	−	−	−	−
S11(L1)	−	−	+	+	−	−	−	−	−	−	−	−
S11(L2)	−	−	+	+	−	−	−	−	−	−	−	−
S11(L3)	−	−	+	+	−	−	−	−	−	−	−	−
S34	−	+	−	±	−	−	−	+	+	−	−	−
S34(W2)	−	−	−	+	−	−	−	+	+	−	−	−
S34(W4)	−	−	−	−	−	−	−	+	+	−	−	−
S34(M3)	−	+	−	−	−	−	−	−	−	−	−	−
S34(M4)	−	+	−	−	−	−	−	−	−	−	−	−
S34(L2)	−	−	−	−	−	−	−	+	+	−	−	−
S13	+	−	+	+	+	+	+	+	+	+	+	−
S13(W1)	−	−	+	+	−	+	+	+	+	+	+	−
S13(W3)	+	−	−	−	+	+	+	−	−	+	+	−
S13(W4)	−	−	−	−	−	−	−	+	+	−	−	−
S13(W5)	−	−	−	±	−	−	−	+	+	+	−	−
S13(M1)	−	−	±	+	−	−	−	+	+	−	−	−
S13(M2)	−	−	+	+	−	−	−	+	+	−	−	−
S13(M3)	−	−	−	−	−	−	−	+	−	−	−	−
S13(M4)	+	+	−	+	+	−	+	+	−	+	+	+
S13(L1)	+	−	+	+	+	+	+	+	+	−	−	−
S13(L2)	+	−	+	+	+	+	+	+	+	−	−	−
S45	−	+	−	−	−	−	−	−	−	−	−	−
S45(W2)	−	−	−	−	−	−	−	−	−	−	−	−

biological affinities. Bacteriophages W3, W4, M3 and M4 constituted a well defined group only tenuously related to W2. L1, L2 and L3 were very similar to each other and to W1, W5 and M1. M2 and W2 were less closely allied with this group. The relationship among these bacteriophages is shown in FIG. 2.

The hosts employed were also related. Although 4 different species were used, hyphal fusion has been demonstrated between *S. griseus* and each of the other 3 species. *S. griseus* strain S86 and *S. venezuelae* strain S13 had identical viral host ranges. The cross resistance patterns of S86 and S13 were different, however. Strains S11, S104 and S86 were closely allied. The biological affinities of the organisms studied may be expressed as a continuous series: *S. venezuelae* strain S13—*S. griseus* strain S86—*S. griseus* strain S104—*S. olivaceus* strain S11—*S. griseus* strain S34—*S. griseus* strain S1954—*S. cyaneus* strain

S45. With respect to viral susceptibility, *S. griseus* strain S86 resembled *S. venezuelae* strain S13 more than *S. griseus* strain S1945.

Selection of S86 resistant to W4 conferred resistance to all other phages tested. A similar situation exists in *Clostridium madisonii* strain 4J9 wherein lysogenization resulted in broad-spectrum resistance (Peri and Watson, **1948**). This provides a reasonable method of developing immune strains of industrially important *Streptomyces*.

Resistant mutants frequently simultaneously developed immunity to several different bacteriophages. Conversely, selection for resistance to one phage sometimes resulted in sensitivity to a second phage. For example, a mutant of S13 resistant to M4 was susceptible to L3 whereas the parental S13 was resistant.

TABLE 1 shows the most frequent classes of bacteriophage-resistant mutants, except for S104 selected for resistance to W3. In all cases other resistance patterns occurred, but less frequently. According to expectation, resistance to M3, M4 and W4 usually developed simultaneously with resistance to W3. Specific mutants, i.e., altered only with respect to W3 susceptibility, were readily obtained.

Resistance could be the result of lysogenization. Bacteriologically sterile autolysates of each resistant strain were placed on lawns of the hosts employed in this investigation. Some of the hosts were known to be lysogenic (Bradley, **1957**). Autolysates of lysogenic strains yielded plaques of the expected type. No other types of bacteriophage were recovered. Resistance was not the result of lysogenization.

Twelve different bacteriophage isolates could be distinguished by host range. This might mean there have been no recurrences or that mutations had occurred in the stock phages leading to the observed diversity.

SUMMARY

Strains of *Streptomyces* selected for resistance to one bacteriophage frequently became resistant to other bacteriophages. Twelve bacteriophages of different origin were arranged into allied groups on the basis of cross resistance patterns. In one instance, resistance to one bacteriophage conferred immunity to all other phages tested. Conversely selection for resistance to one bacteriophage rarely resulted in sensitivity to a second phage. Lysogenization was not responsible for the bacteriophage resistance.

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GENETICS OF COCHLIOBOLUS HETEROSTROPHUS. III. GENETIC FACTORS INHIBITING ASCUS FORMATION [1]

R. R. NELSON [2]

In previous investigations on the genetics of sexual reproduction in the fungi, major emphases have been placed largely upon a determination of the type of sexual reproduction (i.e., homothallic or heterothallic) and evolution of the positive genetic factors conditioning cross- or self-fertility. It is of considerable significance to note that in fungus species in which the genetic systems have been examined extensively, such as *Neurospora tetrasperma*, *Schizophyllum commune*, and *Glomerella cingulata*, a number of genetic factors have been reported to block the completion of the sexual process at various stages in the reproductive cycle (**2**, **4**, **9**, **11**).

Within the Ascomycetes, there are at least 3 specific stages in sexual reproduction at which genetic factors are capable of precluding subsequent development. In heterothallic species, perhaps the most basic inhibition is the failure of nuclei from apparently compatible strains to pair and thus initiate plasmogamy. In recent studies with *Cochliobolus heterostrophus*, it was shown that monoconidial and monoascosporic isolates of both compatibility groups, *A* and *a*, vary in their degree of compatibility in that known compatible isolates frequently fail to produce perithecia when paired (**6**). The genetic mechanism for such variability apparently is controlled quantitatively, although its operation still is not fully understood. A recent cross of strains of *C. heterostrophus* has yielded monoascosporic isolates that exhibit mating inhibition to the compatible parental isolate and to certain wild-type isolates of the opposite compatibility group. A number of mutant genes in *G. cingulata* have been reported to block plasmogamy (**10**). Three

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of the mutant genes control requirements for growth factors, namely arginine, biotin, and thiamine.

The failure of dikaryons to fuse (karogamy) at the expected point in the life cycle has been reported in a number of fungi, including *Itersonilia perplexans* (**8**), *Kunkelia nitens* (**3**), and *Sporodinia grandis* (**1**). In *G. cingulata*, 1 gene, B^2, has been found to affect the fusion of nuclei by causing nuclear disintegration just prior to or during karogamy (**10**).

Inhibition may also occur following the fusion of nuclei. Dodge, working with *N. tetrasperma*, observed that some ascocarps produced asci in which spores were not delimited (**2**). He attributed such ascus abortion to a lethal gene, which was transmitted through subsequent generations in ascospores containing 2 nuclei of opposite compatibility. Uninucleate ascospores carrying the lethal factor died shortly after germination. Genetic factors inhibiting ascospore formation also have been reported in *C. heterostrophus* (**7**). The mechanism conditioning the formation of sterile asci is controlled by a number of genes.

Monoascosporic strains carrying factors for ascus abortion have been used in a series of mating studies with wild-type isolates. From 3 such crosses, ascospore progenies have been obtained that produce sterile perithecia when selfed. The results of the studies with these isolates are reported herein.

MATERIAL AND METHODS

The materials and methods used in these studies were similar to those described previously (**5, 7**). Compatible isolates were mated by placing small pieces of mycelium on opposite sides of a section of sterile corn leaf placed in the center of a Petri plate containing Sach's nutrient agar. All matings were incubated continuously at 24° C.

Sixty-three ascospore isolates were obtained from a cross of a "sterile-ascus" strain and a wild-type, conidial strain isolated from diseased corn leaves collected in western Virginia. The compatibility reaction of the ascosporic isolates was determined by mating each isolate to both parental strains and to *A* and *a* tester strains. Several of the 63 ascospore isolates, selected at random, then were crossed with other compatible conidial isolates. Ascospores obtained from these crosses were selfed and mated to tester lines for compatibility reaction. Three of the isolates, designated as S1, S2, and S3, produced perithecia in selfed cultures and in matings with both tester strains. Upon further examination, it was found that the perithecia contained no asci. Subsequent investigations indicated that the 3 sterile isolates behaved similarly. The results summarized in this paper are concerned only with S3.

RESULTS

Mature perithecia of *C. heterostrophus* are subglobose to ellipsoidal and usually measure from 350 to 650 μ in transverse and vertical diameter. Perithecia are characterized by the formation of a well-defined, somewhat tapered ostiolate beak, ranging in length from 100 to 400 μ. Most of the sterile perithecia produced in selfed cultures of isolate S3 differ in appearance from normal perithecia in that the beak is generally shorter, somewhat thickened, and poorly developed. Such perithecia frequently appear abortive and poorly developed. However, some of the sterile perithecia produced by S3 are similar in appearance to normal perithecia in all respects.

The production of sterile perithecia by isolate S3 could be maintained by mycelial transfers of the original culture or from Petri plates of subsequent generations of the isolate. The mycelial transfers, referred to as "agar-block" transfers, constituted $\frac{1}{4} \times \frac{1}{4}$ inch blocks of mycelial growth and agar. All agar-block transfers of S3, taken from 12 successive vegetative generations, have produced sterile perithecia. Similar results were obtained with 100 hyphal-tip isolates and 50 monoconidial sub-isolates of S3 cultured through 3 vegetative generations. The fact that dissociation did not occur indicated that isolate S3 is homocaryotic

All efforts to obtain fertile crosses of S3 with other isolates of *C. heterostrophus* have been unsuccessful. Attempted matings included pairing S3 with approximately 60 conidial isolates representing a wide geographic and genetic base, 30 ascospore isolates obtained from the same cross that yielded S3, and a number of genetic variants, including albino isolates, monoascosporic isolates inhibited to their compatible parental strain, and isolates carrying factors for ascospore inhibition. Only 4 poorly developed asci were observed in several hundred perithecia produced in paired cultures.

In previous studies with *C. heterostrophus,* particularly those concerned with variability in degree of compatibility (**6**), the position of perithecium formation in relation to the position of the isolates involved in the cross was found to be a useful criterion in determining the type and degree of interaction between isolates. In most instances, the rate and pattern of growth made it possible to ascertain which isolate of the mating produced the perithecia. In the present investigations, similar observations were made in paired cultures of S3 and *A* and *a* wild-type isolates. In paired cultures, most of the perithecia that formed on the S3 side of the leaf developed short, thickened, poorly defined beaks, typical of the sterile perithecia in selfed cultures of S3. However, nearly

all of the perithecia produced on the side of either the *A* or *a* isolates were normal in appearance, although sterile. Since normal heterothallic isolates do not produce perithecia or perithecial initials when selfed, the results demonstrated a degree of interaction between S3 and other isolates. With respect to compatibility reaction, this interaction suggested that isolate S3 was capable of functioning either as *A* or *a* mating type, at least to a certain extent.

The possibility that a diffusible substance was responsible for ascus inhibition in paired cultures was investigated. Known compatible strains were paired on opposite sides of a corn leaf on Sach's nutrient agar. A cellophane disk, the diameter of a Petri plate, was placed on top of the paired cultures. A mycelial transfer of S3 was placed on the cellophane directly over the corn leaf. All perithecia produced by the paired strains were fertile.

Agar-block transfers of S3 also were placed on adjacent sides of a paired mating of compatible isolates. Nearly all of the perithecia produced in the vicinity of the compatible isolates were normal, while perithecia produced near the transfers of S3 were sterile and typical in appearance of perithecia of S3 produced in selfed cultures. Ascospore progenies obtained from the fertile perithecia were heterothallic.

Similarly, no inhibition was obtained when a cell-free filtrate of S3 was added to Petri plates of agar supporting known compatible pairings.

DISCUSSION

The production of normal-appearing, although sterile, perithecia in paired cultures of S3 and *A* or *a* isolates, as well as the production of perithecia in selfed cultures, suggests that isolate S3 has all of the genetic components for both compatibility groups. The continued stability of hyphal-tip progenies and monoconidial cultures of S3 demonstrates that all of the factors are carried within a single nucleus. It may be assumed that the failure of asci to form in perithecia in selfed or paired cultures is governed by the same genetic mechanism.

The inability to cross the "sterile-perithecial" strain with normal heterothallic isolates has precluded any genetic analysis of the factors inhibiting ascus formation. Without such information, it is difficult to speculate as to the origin of isolate S3. In view of the fact that the sterile strains were obtained as second-generation ascospores from a cross involving an isolate carrying factors for ascospore inhibition, it is possible that the mechanisms governing sterile perithecia and sterile asci have 1 or more genes in common.

In a previous publication concerned with the genetics of *C. heterostrophus*, it was stated that little is known about the genetic complex that differentiates homothallism from heterothallism (**7**). It was postulated that perhaps some heterothallic species are self-sterile not because they lack the necessary genes for sexual reproduction but because of the presence of genes that block specific steps in reproduction. The ability of certain progeny, obtained from crossing what are considered to be heterothallic strains, to produce sterile perithecia when selfed would appear to represent a basic step in a transition from heterothallism to homothallism.

At the present time, it is not known at what stage in the sexual process inhibition occurs. Two poorly developed asci and several structures resembling ascus croziers have been observed in perithecia produced in paired cultures of S3 and normal heterothallic isolates. Three immature asci also have been observed in perithecia produced in selfed cultures of S3. These observations would indicate that plasmogamy occurs in at least some instances. The production of greater numbers of normal-appearing perithecia in paired cultures also might indicate the initiation of the dicaryophase. In either case it is not likely that nuclear fusion occurred, although detailed cytological studies are necessary to determine nuclear behavior. Such studies with this and other genetic variants are in progress.

It should be pointed out that the present postulation regarding the genetic basis for heterothallism is proposed merely as a possible explanation. The ability to obtain "sterile-perithecial" strains and "sterile-ascus" strains from a supposedly heterothallic species may have an entirely different genetic explanation. The real importance of the studies to date lies in the fact that such genetic variants have been obtained.

SUMMARY

A monoascosporic isolate obtained from a cross of a wild-type conidial strain and an isolate carrying factors for ascus abortion produced sterile perithecia when selfed. Most sterile perithecia differed from normal mature perithecia in the formation of a short, thickened, poorly developed beak, although some sterile perithecia were similar to normal perithecia in all respects.

Transfers from the original cultures and sub-cultures thereof, as well as hyphal-tip progenies and monoconidial sub-cultures, consistently produced sterile perithecia through 12 successive generations, indicating the strains to be homocaryotic.

Although no fertile crosses of the "sterile-perithecial" strain and

wild-type isolate have been obtained, the production of greater numbers of normal-appearing perithecia and occasional immature asci and ascus croziers indicate a degree of interaction between the sterile strain and normal isolates. It is suggested that the sterile strain may function as either mating type.

There appears to be a specific relationship between the genetic mechanisms governing ascus inhibition and ascospore inhibition.

It is postulated that the ability of a monoascosporic isolate to produce sterile perithecia in selfed cultures represents a basic step in a transition from heterothallism to homothallism.

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EFFECTS OF WOOD AND WOOD PRODUCTS ON PERITHECIAL DEVELOPMENT BY LIGNICOLOUS MARINE ASCOMYCETES

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Since the pioneer studies by Barghoorn and Linder (**1944**), an increasing number of papers have been published dealing with taxonomy and, to a lesser extent, distribution and abundance of lignicolous marine fungi (Johnson and Meyers, **1957**). Few investigations have been concerned specifically with the physiological aspects of these fungi. Barghoorn and Linder reported on physiological studies of selected species of marine fungi, and examined the effects of different media, including cellulose and wood flour, on vegetative growth. The mycelial growth rates and mode and rate of perithecial production on sea-water agar of seven different lignicolous Ascomycetes were studied at this laboratory (Meyers, **1954**). Recently, Gustafsson and Fries (**1956**) investigated the nutritional requirements of various marine species of Ascomycetes and Deuteromycetes and recorded the rate of mycelial development on differential media. To our knowledge, the report from this laboratory (**1954**) was the first paper noting the development of perithecia in cultural studies of marine lignicolous Ascomycetes.

During the past several years, we have been studying the physiology of these fungi, including nutritional studies, as well as tests in which wood and wood constituents are used to elucidate specific metabolic activities, such as reproduction. The latter work is reported here. The results of minimal-nutrition tests will be presented in a subsequent paper.

MATERIALS AND METHODS

The fungi used in this study were *Lulworthia grandispora* Meyers, *L. floridana* Meyers, *L. medusa* var. *biscaynia* Meyers, *Antennospora quadricornuta* (Cribb and Cribb) Johnson, *Ceriosporopsis halima* Linder, *Lignincola laevis* Höhnk, *Torpedospora radiata* Meyers, *Peritrichospora*

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integra Linder, and *Arenariomyces salinus* Meyers. These genera correspond respectively to Forms Nos. 1–7, collected from wood panels submerged in the sea (Meyers, **1953, 1954**). All of the cultures were grown originally from single-spore inoculations. *L. grandispora, L. floridana,* and *L. medusa* var. *biscaynia* initially comprised a group of isolates from twelve geographic regions. Cultural characteristics and perithecium and ascospore dimensions were used to establish species and varietal relationships (Meyers, **1957**).

Stock cultures of the marine species were grown on sea-water-agar slants containing 0.1% yeast extract (Difco), 1.0% glucose and 1.5% agar. In the early phases of this work developed at Columbia University, the sea water was taken from the Atlantic Ocean off Long Island, New York. Subsequent sources at Miami, Florida, came from Biscayne Bay or the Gulf Stream. We saw no differences in fungal growth on media prepared with sea water from these natural sources. In this series of studies the agar and broth culture media were made with natural sea water and 0.1% yeast extract, unless stated otherwise.

In some of our investigations, an artificial sea water, the ASP_2 medium of Provasoli et al. (**1957**), was used. This medium was supplemented with 0.1% yeast extract or with a nutrient solution containing 0.04% L-asparagine, 0.1% NaH·glutamate, 0.1 mg/100 ml thiamine, and 0.5 μg/100 ml biotin. Glucose (1.0%), either sterilized with the medium or added aseptically after separate sterilization, was supplied as a carbon source. More extensive mycelial development occurs when glucose is added aseptically, after separate sterilization. In certain experiments, various powdered celluloses were substituted for the glucose. The celluloses used were: Alphacel (Nutritional Biochemicals Corp.); Whatman ashless cellulose powder, Standard Grade (for chromatography); and Rayocord-X (F), a southern-pine sulfite pulp (Rayonier, Inc., New York City). Wood flours, used as substitutes for glucose, included balsa, aspen, sugar maple, fir, sweet gum, birch, hemlock and yellow pine. The balsa wood was used both in powder and in regular wood strip form.

None of the wood flours, except yellow pine, were chemically treated. The yellow pine flour was extracted with various solvents, i.e., chloroform, benzol and absolute alcohol, followed by thorough washing with hot and cold distilled water, to remove, insofar as possible, the resins and terpenes.

In the liquid culture experiments, 40–50 ml of a 0.1% yeast-extract–sea-water broth were dispensed into 250 ml Erlenmeyer flasks. Both

stationary and shake cultures were run, the latter on a reciprocating shaking apparatus. One series of flasks was given vigorous sterile aeration. Powdered cellulose or balsa wood pieces and flour were used as sources of carbohydrates. In the flasks with balsa wood pieces, the latter were slanted, with the lower portion immersed in the medium. This arrangement permitted the absorption of liquid throughout the wood, and provided a submerged and aerial surface for subsequent fungal development.

The inoculum for the agar and liquid cultures consisted of mycelial and agar clumps or ascospore suspensions. The latter were obtained by aseptically macerating fructifications of the test fungus in sterile sea water in sterile microculture depression slides. Cultures were incubated at the room temperature of approximately 20–25° C.

The rate and intensity of mycelial development and the extent of perithecial production were used as criteria for estimating the utilization of the different metabolites. Since our objective in this work was to study some of the parameters for reproduction in these species, quantitative measurements were not made. The vigor of perithecial formation on the various substrates was noted in arbitrary terms, ranging from colonies with no sporulation to those with heavy concentrations of perithecia.

In this paper, the terms "reproduction" and "sporulation" are used interchangeably, both referring specifically to the development of perithecia.

RESULTS

The species studied are not fastidious in their nutritional requirements for mycelial development. Excellent vegetative growth occurred in the yeast-extract-glucose medium, prepared with either natural or artificial sea water. Growth in liquid media generally is more rapid than that on agar, especially for *Antennospora quadricornuta*, *Lignincola laevis* and *Arenariomyces salinus*.

The combination of 0.04% L-asparagine, 0.1% NaH·glutaminate, 0.1 mg/100 ml thiamine and 0.5 μg/100 ml biotin, with 1.0% glucose, in conjunction with either natural or artificial sea water, supports the vegetative stage of all of the fungi examined. However, reproduction on this synthetic medium occurs infrequently, even in *L. floridana*, a species characterized by extensive perithecial production on non-synthetic media. The perithecia of *L. floridana* often are atypical and immature on media prepared with artificial sea water, even when 0.1% yeast extract is substituted for the nitrogen-vitamin mixture listed above.

The species of *Lulworthia*, and *C. halima*, *T. radiata*, and *P. integra* all produce maturing perithecia on agar media prepared with natural sea water. Following the initial isolation of these species from submerged wood, and for as many as 5–6 successive generations, perithecial production occurred in colonies on a medium of sea-water agar with no nutritive supplement. Various cellulosic materials, i.e., wood flours or powdered cellulose, in conjunction with 0.1% yeast extract, support reproduction in these species.

Often, the two powdered celluloses tested have dissimilar effects on vegetative growth and sexual reproduction. In one series of experiments, *L. floridana* and *T. radiata* were grown in yeast-extract broth fortified with 1.0% Alphacel or Whatman cellulose. Within 3 weeks, *L. floridana* produced 3–4 times as many mature perithecia in the medium with Whatman cellulose as in that with Alphacel. The amount of mycelial development was approximately equal in both cellulosic media. *T. radiata* showed no difference in the amount of reproduction on these two substrates, although Whatman cellulose supported at least 3 times as much mycelial growth as did Alphacel. A growth factor stimulatory to perithecial formation has been reported present in Whatman No. 1 filter paper (Basu, **1947**).

In contrast to the sporulation in agar culture of the above species, colonies of *Antennospora quadricornuta*, *Lingnincola laevis* and *Arenariomyces salinus* show poor reproduction or none at all in such cultures. The addition of wood flours or powdered cellulose along with yeast extract or various amino-acid and vitamin combinations failed to induce perithecial development in *Antennospora quadricornuta* without further treatment, such as noted below. Mature perithecia of *Lignincola laevis* were produced in several cultures of aspen-wood-flour agar, with and without the presence of yeast extract. In *Arenariomyces salinus*, perithecia were absent on aspen agar, although present in large numbers on yellow pine agar, both media without yeast extract. The fructifications on the yellow pine medium failed to develop mature asci. An accompanying plate of cellulose-yeast-extract agar showed numbers of fertile perithecia, although subsequent cultures of the fungus on this medium produced numerous young fructifications with incomplete ascospore development.

As noted, various wood flours were incorporated into sea-water–agar media, alone or in combination with the yeast extract. Of these woods, aspen and yellow pine were most effective in supporting perithecial production in certain of the species examined, especially *L. grandispora*, *L. medusa* var. *biscaynia*, *L. floridana*, *C. halima*, and *P. integra*. In

the latter fungus, perithecia usually developed on the side of the petri dish at and above the periphery of the agar medium. Reproduction in other areas on the plate could be stimulated by the removal of small blocks of the agar. Within 1–2 weeks, mature perithecia were present in large concentrations on the glass surface exposed by the removal of the agar. Buston and Rickard (**1956**) observed an increased production of perithecia by *Chaetomium globosum* in the region of a physical barrier which impeded the growth of the colony. This was attributed to a locally increased concentration in the medium of organic phosphates excreted by the fungus.

Balsa-wood sections were tested to determine if a natural wood substrate in liquid culture could stimulate sporulation in these lignicolous species, especially those that reproduced poorly or not at all on agar. Autoclaving of the balsa pieces in the yeast-extract broth does not noticeably affect the pH of the medium. However, sterilization of balsa flour under similar conditions adversely modifies the pH so that mycelial development is not extensive. Basswood, birch and yellow pine pieces, when autoclaved in the culture medium, all drastically reduce the pH, this being inhibitory to the growth of these marine fungi. However, these 3 woods have been excellent substrates for the collection of marine fungi in our field studies.

All of the species exhibited abundant mycelial growth upon the aerial and submerged surfaces of the balsa wood in the flasks of yeast-extract broth. Reproduction was common in the culture of *L. floridana, L. grandispora, L. medusa* var. *biscaynia, C. halima,* and *T. radiata,* occurring on both the aerial and submerged wood surfaces. In *Antennospora quadricornuta, Arenariomyces salinus* and *Lignincola laevis,* perithecia were produced slightly or not at all, although mycelial growth of the fungi occurred over the wood. Nevertheless, mature fructifications developed when this fungus-infested wood was transferred to aquaria of aerated sea water. Under these conditions, vigorous reproduction occurred on all surfaces of the wood, often within 2–3 weeks. New pieces of wood, including balsa, basswood, and yellow pine, placed adjacent to the infested wood in the aquaria, showed infestation and sporulation by these species within several weeks.

DISCUSSION

The use of natural plant materials to enhance the development of various terrestrial fungi in culture is common procedure. Buston and

Basu (**1948**) found that a hot-water extract of jute fibre, *Corchorus capsularis,* stimulated the growth of and accelerated sporulation in *Chaetomium globosum.* Various workers (Robbins and Ma, **1942**; Zuck and Diehl, **1946**) have reported the beneficial effect on fungal growth of the addition of cotton extracts to nutrient media. Often, the extract furnished vitamins required by the particular fungus. Recently, Robbins et al. (**1955**) observed a marked stimulus in the growth of *Stereum murraii* by extracts from beech, *Fagus sylvatica,* or birch, *Betula lenta.* Thiamine or its equivalent and available nitrogen were demonstrated to be present in the wood extract. In our studies with marine fungi, it has been noted that the addition of small amounts of a hot-water extract of ground basswood to sea-water agar stimulated spore germination in many species. Similarly, the presence of the soluble components from the balsa wood in the yeast-extract-broth cultures enhanced ascospore germination.

In our experiments, we have attempted to approach, as closely as possible, the natural conditions under which these lignicolous fungi are found. Barghoorn (**1944**) notes, "The fungi under natural conditions very commonly become established on material covered by surface growths of various benthonic organisms, presenting upon decomposition a readily available supply of organic nitrogen." Thus it is not surprising that excellent reproduction has occurred on wood in sea-water-broth cultures, supplemented with yeast extract.

It should be noted that both perithecial production and vegetative growth may be favored by the same conditions, but not the completion of the sexual process. Wood cultures produce the conditions favoring all three processes, both on panels submerged in sea water and in laboratory cultures. The reasons for the failure of various species to form fertile perithecia when artificial sea water is used are not apparent. Possibly an imbalance in the components of the medium, or the absence of some, adversely affect the maturation of asci and ascospores, since they are the resultant of the sexual process.

While a tabulation of the vegetative growth of the fungus on culture media is important, the completion of the sexual stage permits a more definitive analysis of the nutritional requirements of the organism, as well as a better understanding of the physical and chemical determinants of reproduction.

Our studies on the effect of wood and wood products on perithecial production are being supplemented by work on the presence of wood-destroying enzymes in these lignicolous species.

ACKNOWLEDGMENTS

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SUMMARY

Studies are reported in which wood and wood products, incorporated into culture media, have been used to stimulate perithecial production in seven genera of lignicolous marine Ascomycetes. The species examined are not fastidious in their requirements for mycelial growth. Fertile fructifications are produced in cultures grown on balsa-wood pieces partly submerged in flasks of yeast-extract broth. Differences in the intensity of perithecial development and in the conditions necessary for reproduction are noted for the various genera. Media with powdered cellulose or wood flours, supplemented with 0.1% yeast extract, support sporulation in certain of the species.

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LYOPHILIZATION OF THE ACTINOPLANACEAE[1]

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When the Actinoplanaceae were first described (Couch, **1949, 1950**) only thirteen isolates were in our collection. Today there are over five hundred and the number is constantly increasing. In addition a stock collection of approximately one hundred closely related organisms is maintained. These organisms are being studied taxonomically and in other ways and it is essential that their viability be maintained. Furthermore, it has been noticed many times that some of the Actinoplanaceae undergo mutations in artificial culture so that it is very difficult to maintain cultures with the characters shown by the original isolates. For example, a considerable number of our collections have apparently lost their ability to form sporangia. The experiences of other workers, Wickerham & Andreasen (**1942**), Raper & Alexander (**1945**), Fennell, Raper, & Flickinger (**1950**) and Haynes, Wickerham, & Hesseltine (**1955**), with other organisms have shown in many cases that the lyophile process is the most reliable means of culture maintenance. This is a brief report of our experience with lyophilization of the Actinoplanaceae.

APPARATUS AND METHODS

The lyophile apparatus used in our laboratory consists of a stainless-steel freeze-dryer with fourteen ports, two of which are for connections, a stainless-steel trap, a McLeod vacuum gauge, and a vacuum pump. A gas-oxygen torch with a "Y" tip is used to seal the tubes.

A relatively simple method requiring the minimum of special preparation and equipment was devised and was found to work excellently for preservation of the Actinoplanaceae by the lyophile process. A 10 in. length of pyrex glass tubing, 9 mm outer diameter and 7 mm inner diameter, is heated in the middle; the two portions are drawn apart and sealed. The open end of the tube is then fire-polished. Two-tenths ml of suspending solution, Difco dehydrated skim milk, prepared

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as directed on the container but double strength, is put into each lyophile tube. Tubes thus prepared are autoclaved for fifteen minutes at fifteen pounds pressure. The end of a 1.5 in. piece of chromel wire is fashioned into a spatulate shape and the other end is attached to a loop-holder, thus making a transferring tool.

Three- to four-weeks-old, regular stock tubes of Actinoplanaceae growing on peptone-Czapek medium are used as inocula. This medium causes the mycelium of most Actinoplanaceae to "heap up" thus making it easy to avoid the transfer of much agar. The transferring tool is flamed, dipped in 95% ethanol, flamed again and then used to transfer the mycelium from the stock tube to the lyophile tube. The inoculum is inserted all the way down the narrow lyophile tube into the suspending solution, and while arranged thus, the transferring tool is rotated back and forth rapidly. The spatulate-shaped end with the inoculum on it, as it rotates back and forth, disperses the inoculum in the suspending solution. The tubes are then numbered and dated.

Twelve tubes prepared as above are placed in the freeze-dryer simultaneously in dry ice and solvent (95% ethanol) at a temperature of −60° C for ten to fifteen minutes. After a vacuum of 100 μ or less is achieved in the freeze-dryer, the tubes with the frozen suspending solution and dispersed inocula are inserted one by one in the ports of the freeze-dryer and dried at room temperature. Usually a vacuum of 50 μ to 5 μ is maintained; successful freeze-drying was occasionally done at vacuums considerably less than this (500–400 μ). The contents of the tubes are vacuum dried usually one and one-half to two hours. They are then sealed under vacuum with a gas-oxygen torch. Usually within twenty-four hours, the lyophile preparations are tested with a high-frequency spark-coil tester to determine whether or not a vacuum is present. Completed preparations are then stored in flat boxes in the refrigerator at 6°.

The lyophile preparations were tested at 3- to 4-, 4- to 5- and 6- to 7-month intervals. The top of the lyophile tube was broken off, the pellet shaken on a peptone-Czapek-agar plate, and 2–3 ml of sterile distilled water was placed on the dry pellet.

Experiments to determine the part or parts of the organism which survive the freeze-drying process were set up. Eleven isolates, started from a single sporangium or a minute point inoculum, were cultured on peptone-Czapek medium in petri dishes and allowed to grow for ten days. The entire mycelial growth, which at this time in most cases measured only 1–2 mm in diameter and which had not produced sporangia, was put into lyophile tubes and lyophilized. The viability of

these isolates was checked in thirty days. Sporangia were carefully scraped from eight different isolates growing on Czapek medium. This was done with the aid of the dissecting microscope to be reasonably sure that only sporangia were removed. The sporangia were then lyophilized as described above. The viability of these isolates was checked in seven days.

RESULTS

Of 326 lyophile preparations tested, including in this number 266 different isolates, all were found viable. In addition, the vitality of the isolates was apparently not impaired in any way. Growth occurred from vast numbers of pieces of mycelium, both microscopic and macroscopic in size.

Results of the experiments to determine if the mycelia and sporangia will withstand freeze-drying indicated that they do survive the process, for all of the isolates used in these experiments were found to be viable.

DISCUSSION

How significant are the viability tests made just three to seven months after the isolates have been lyophilized? Fennel et al. (**1950**) state that viability tests made immediately following lyophilization are significant. They ". . . consistently observed during an eight year period that once cultures have been lyophilized successfully, they retain their viability with little or no apparent decline. When difficulties are experienced, they are encountered during the freezing and drying process." Since we must transfer our cultures every six to eight weeks—some of them every three to four weeks—while carrying them on nutrient-agar slants, such excellent viability after three to seven months is considered significant.

It has been suggested that a large proportion of the cells are killed by freeze-drying and that this selective killing might cause variation in lyophilized cultures. Haynes et al. (**1955**) concluded that variation and mutation in lyophilized cultures is not a problem. In their experience with the organisms in the Northern Utilization Research Branch culture collection, this has not happened in lyophilized preparations. Fennel et al. (**1950**) have found that in a period of eight years no colonies of conidium-producing molds which have developed from lyophilized spores differed in any detectable degree either morphologically or physiologically from those developing from non-lyophilized spores of the same strain. While a thorough investigation of the effect of lyophilization on variation in the Actinoplanaceae was not made, the results from the viability

tests indicate that very few if any mycelia of the isolates were killed by the freeze-drying process. This suggests that the freeze-drying process does not produce variation by selective killing in this group. In the few cases where only a small amount of the mycelium survived, it was found that this could be attributed to the old age of the culture when it was lyophilized. Usually it is best to lyophilize cultures of the Actinoplanaceae when they are three to four weeks old, since many of them growing on peptone-Czapek medium begin to undergo lysis after six weeks or so.

These organisms are handled perhaps more in the manner of the lower bacteria than as most mycelium-producing organisms, which are usually not preserved by the freeze-drying of vegetative structures. The Actinoplanaceae are propagated in the main by bits of vegetative growth, which can easily be produced by agitation of the mycelial inoculum in the suspending solution by the above described method. Thus it is not necessary to process the mycelium in a tissue grinder. In addition, the methods described above reduce the chances for contamination, as tubes are only entered once, and no special preparation is necessary, as regular stock tubes may be used as a source of inoculum.

The results of the above experiments indicate that sporangia as well as mycelia can survive the freeze-drying process. In the lyophile preparations of the stock tubes reported above it is thought that in most cases it is the mycelium which has been lyophilized, for isolates used as inocula and growing on peptone-Czapek slants produce much vegetative growth but few sporangia within three to four weeks.

SUMMARY

The methods and apparatus used for lyophilization of the Actinoplanaceae are described. One hundred per cent viability of 326 lyophile preparations, including in this number 266 different isolates, indicates that freeze-drying is a most reliable means of maintaining cultures of the Actinopalanaceae. The results of experiments show that both the mycelium and the sporangia can survive the freeze-drying process.

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SEXUALITY IN PHYSODERMA PULPOSUM WALLROTH

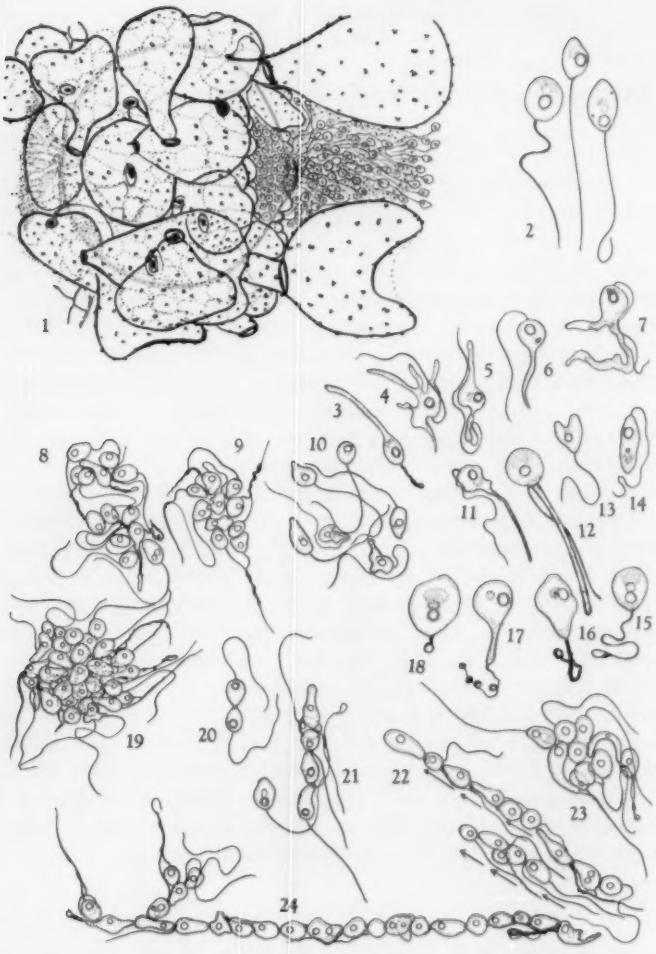
YAMUNA LINGAPPA

(WITH 55 FIGURES)

The occurrence of sexual reproduction and development of the endobiotic phase from zygotes have been reported briefly in *Physoderma pulposum* Wallroth (**5**). A detailed description of the origin, behavior and fusion of the gametes is given below. The same material and methods described previously by the author (**5**, **6**, **7**) for obtaining the planospores and gametes were used in this study. Over-ripened but viable epibiotic sporangia were used as the source of gametes. Usually, after maintaining the inoculated *Chenopodium album* and *Atriplex patula* plants in the greenhouse at 20–25° C for 8–12 days, shoots bearing abundant epibiotic sporangia were cut and stored in closed containers at 5°. Single or several sporangia could be easily dissected, as desired, from fresh or stored plant parts. When placed in charcoal-treated water, the ripe epibiotic sporangia discharge planospores in 10–30 minutes (FIG. 1). The general procedures for obtaining sporogenesis and spore discharge, and for observation of the activity of planospores, gametes and zygotes, were essentially the same as those described for *Synchytrium brownii* by B. T. Lingappa (**4**). These involved the study of planospores from a single isolated sporangium as well as of mixtures of planospores from two or more sporangia. As the planospores derived from germinating resting sporangia did not function as gametes, the following account relates to the activities of the planospores derived from the epibiotic sporangia.

OBSERVATIONS

The planospores and gametes vary from 2.5–5 × 3–7 μ, and frequently multiflagellate giant planospores occur which measure 16 × 8 μ or up to 20 μ in diameter (FIGS. 25–27). In mixtures of planospores derived from different sporangia, the individuals swim about actively and aimlessly at first. Sooner or later, however, depending on room temperature and maturity of the sporangia from which they were derived, some of them begin to follow one another and form actively



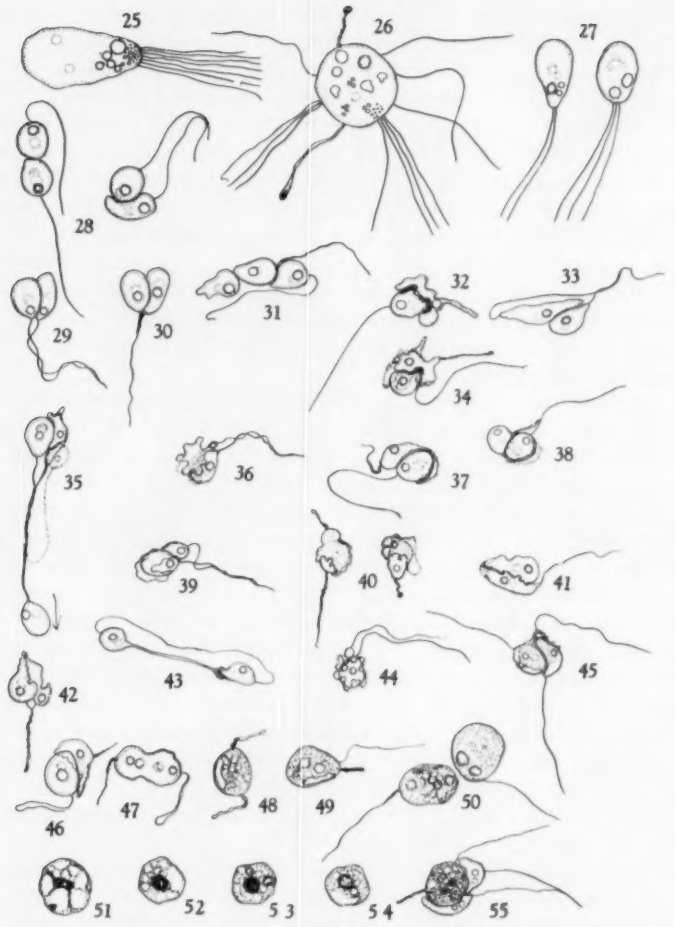
FIGS. 1–24.

FIGS. 1–24. *Physoderma pulposum* on *Chenopodium album*. Planospores derived from epibiotic ephemeral sporangia. Drawn from living preparations. FIG. 1. Emergence of planospores. × 450. FIG. 2. Newly emerged planospores. × 1500. FIGS. 3–7. Planospores on amoeboid condition. × 1500. FIGS. 8, 9. Aggregates of planospores. Flagella of some planospores are entangled. × 750. FIG. 10. Amoeboid inactive and normal active forms in hanging drops containing

swimming pairs (FIGS. 28, 29), small or large groups, or clumps (FIGS. 8, 9, 19) and long chains (FIGS. 21–24). The remainder continue to swim about singly for about three hours and settle down singly. They retain their flagella when they become sedentary, and apparently function as zoospores and may be interpreted as sexually neutral. On the other hand, planospores which follow one another and pair are considered as potential gametes. Whenever small gametes pair with large ones, the pairs suggest heterogamy (FIGS. 33–35, 39) but there is no heterogamy because the size is not related to sex of the gametes. Pairs may separate after a few minutes, or two hours after coming together. Similarly, one or two individuals may join, or separate away from clumps and chains. If the pairs continue together and ultimately fuse, they are referred to as stable pairs as compared with those which are associated temporarily.

In stable pairs one of the gametes ultimately becomes sedentary and usually gradually absorbs its flagellum (FIGS. 32, 38, 41), while the other one remains active for a longer time. In this condition the active gamete continues to push against the quiescent one (FIGS. 34–46), but steadily loses activity and becomes sedentary. Its flagellum remains intact, whereas the flagellum of its partner is being absorbed (FIGS. 34, 37, 38, 46). Sometimes, however, both gametes of a pair may retain their flagella after becoming sedentary (FIGS. 42–46). Also, among the free-swimming single planospores the flagella of some individuals are partially absorbed, and they are approached or followed by active flagellate planospores. Accordingly, the planospores of *P. pulposum* at this stage may be regarded, for convenience, as of two types on the basis of their behavior, namely a sluggish or sedentary "receptive" and an active "searching" type. The latter retains its flagellum even when sedentary while the former usually absorbs the flagellum shortly after becoming sedentary. Thus, the stable pairs consist at the time of fusion of flagellate and aflagellate gametes (FIGS. 40, 41). In a 6-hour-old mixture of planospores at 20° all degrees of active motility, sedentation, flagellar absorption, pairing and fusion may be found. This indicates that not all planospores undergo the same degree and rate of differentiation.

separate single sporangia. × 750. FIGS. 11–14. Different forms of planospores, 6 hours after emergence. × 1200. FIGS. 15–18. Stages of absorption of flagella, in sedentary spores. Seen under light microscope. × 1500. FIG. 19. Large clump in which individuals are sedentary. × 750. FIG. 20. Pair of planospores pushing against each other. × 750. FIG. 21. Chain of planospores separating away. × 750. FIG. 22. Chains of planospores in fast unidirectional movement. × 750. FIG. 23. Short chain of planospores surrounded by stray planospores and forming a cluster. × 750. FIG. 24. Branched chain of planospores, temporarily quiescent. × 750.



FIGS. 25–55.

FIGS. 25–55. *Physoderma pulposum* on *Chenopodium album*. Planospores derived from over-ripe epibiotic sporangia. (FIGS. 51–55. Drawn from stained preparations.) FIGS. 25–27. Giant planospores. × 1000. FIGS. 28, 29. Pairs of active gametes. × 1200. FIG. 30. Quiescent pair. Flagellum of one has fused with that of the other. × 1200. FIG. 31. Chain of planospores, one of them amoeboid. × 1000. FIGS. 32–34. Sedentary pairs, each consisting of a quiescent and an active gamete. × 1000. FIG. 35. Stray active planospore entangled with sedentary pair.

In contrast to the behavior of planospores derived from several sporangia noted above, planospores from a single sporangium neither pair nor fuse. So far, more than one hundred single sporangia were mounted in separate hanging drops and the behavior of the planospores was studied from the time of emergence to degeneration. These planospores remain actively swimming for 4–8 hours at 24°, and up to 72 hours at 5°, but in no instance were pairing and fusion of gametes observed. In this connection it may be mentioned that when mixtures of planospores from several epibiotic sporangia were used as inocula both epibiotic sporangia and the endobiotic phase develop on the host. On the other hand, if planospores from a single sporangium, or from germinating resting sporangia, are used as inocula only epibiotic sporangia develop on the host. As the author (**5, 6, 7**) has pointed out, the epibiotic phase develops from the unfused planospores in such inocula whereas the endobiotic phase develops from zygotes.

COPULATION

As noted above, potential gametes differentiate into sedentary and active types (FIGS. 10, 15–18, 40, 41), and these can be best observed in separate pairs, chains, or small clumps. When the undifferentiated gametes pair, they swim together (FIGS. 28, 29) for an hour or more at 20–25°. During this activity one of the gametes of a stable pair gradually becomes sluggish and both begin to stop and swim intermittently. As time passes, quiescent periods become longer and more frequent. When temporarily quiescent, the less active gamete becomes amoeboid and its pseudopodia overlap the active gamete (FIGS. 34–46), or curve around it (FIGS. 32, 37). After a while, it returns to the original shape and both start swimming again. Later on, however, when both gametes are sedentary, both become amoeboid and envelop, or crawl over, each other (FIGS. 40, 41, 46). The pseudopodia are intermittently retracted and reformed. In this process the pseudopodia from both gametes come into close contact and coalesce, and the contents quickly intermingle (FIGS. 47–50). Plasmogamy is thus completed

× 1000. FIGS. 36–46. Pairs of gametes showing amoeboid stages prior to plasmogamy. × 1000. FIG. 47. Plasmogamy. × 1000. FIG. 48. Biflagellate zygote. × 1000. FIG. 49. One of the flagella of a biflagellate zygote being absorbed. × 1200. FIG. 50. Uniflagellate zygotes. × 1200. FIGS. 51–54. Sedentary zygotes which have absorbed their flagella. × 1000. FIG. 53. Zygote containing two supernumerary haploid nuclei alongside a diploid nucleus. × 1000. FIG. 55. Planospores clustering around a zygote. × 1000.

quickly but only after prolonged activity. At 20–25° differentiation of gametes, subsequent activity and fusion occur 3–8 hours after the planospores emerge from the sporangia.

Variations of the above process occur. Both gametes may retain their flagella so that the zygote is biflagellate (FIGS. 47–49). However, in the majority of cases the gamete which became sedentary first is aflagellate at the time of fusion so that the newly formed zygote is uniflagellate (FIG. 50). In any event, within 10 minutes after plasmogamy the zygote becomes active again and moves slowly for a short period. Then it settles down, becomes subspherical and absorbs the flagellum (FIGS. 51–54). In stained preparations, karyogamy has been found to take place at this stage of the zygote (FIG. 51). At this stage, the zygote is 6–7 μ in diameter with a nucleus 2–2.5 μ in diameter. The two refringent yellow globules also move closer and coalesce, and the membrane of the zygote appears to become more rigid. Shortly after the zygotes settle down, vacuoles appear in the cytoplasm, imparting a characteristic foamy appearance (FIGS. 51–54). The foamy appearance of the zygotes was different from the more dilute and extensively vacuolate contents of degenerating sedentary planospores.

During the brief period of activity of the zygotes active planospores often surround them, but swim away after a while (FIG. 55). Rarely, however, one of the stray planospores may remain with the zygote and ultimately fuse with it. In stained preparations, several zygotes with one diploid and one or more haploid nuclei (FIG. 53) have been observed. Apparently, plasmogamy occurs between zygotes and gametes, but the supernumerary nuclei do not fuse. Under the conditions of this study, the number of fusions observed was small in comparison with the large number of planospores in each preparation. Many stable pairs degenerate without fusing. However, when samples were taken from the inocula on the host plants and examined at regular intervals after inoculation, fusions were found to occur more abundantly. Degeneration of the sedentary spores, pairs and zygotes was not seen in such samples even after 12 hours.

DISCUSSION

According to these observations, sexual reproduction in *P. pulposum* is isogamous, and involves fusion of gametes from two or more sporangia. The gametes which fail to fuse, and do not degenerate, function as zoospores and infect the host. No evidence of change of sex of the gametes, or of the occurrence of relative sexuality, was observed. However,

aggregations of planospores in pairs and small and large clumps occur in the same manner as described by Kusano (**2**), Köhler (**1**) and B. T. Lingappa (**4**) in *Synchytrium fulgens, S. endobioticum* and *S. brownii,* respectively. In *P. pulposum* chains of planospores which swim in one direction also occur frequently. These aggregates occur in darkness as well as in light, and at 5–27°. Under laboratory conditions, however, only a few of the individuals in such aggregates have been found to copulate, and in many aggregates no zygotes at all are visible. The number and size of the aggregates do not increase, although there may be an abundance of free swimming planospores in the vicinity. Therefore, whether or not aggregation is an expression of sexuality is not certain, but this is suggested at least by the lack of its occurrence among the planospores from single epibiotic sporangia or from germinating resting sporangia.

The present observations confirm the suggestion of Sparrow (**9**) that the planospores derived from epibiotic sporangia of *Physoderma* species might function as gametes, copulate and then give rise to endobiotic phase. Sparrow (**10**), however, failed to find fusion in *P. menyanthis,* but more recently he reported (**11**) occasional copulation in *P. lycopii.* Previously, Wilson (**12, 13**) and Scott (**8**) had reported fusion of zoospores derived from the resting sporangia of *P. alfalfae;* but their observations were disputed by Line (**3**). As pointed out by the author (**7**), if meiosis occurs in germinating resting sporangia of *P. pulposum,* all planospores whether derived from germinating resting sporangia or from epibiotic sporangia might be expected to function as gametes. Nevertheless, in *P. pulposum,* failure to find copulation among the planospores derived from resting sporangia, or from single epibiotic sporangia, cannot be explained satisfactorily at present. At least one factor, namely, over-ripening of the epibiotic sporangia for at least 3 days after maturation, is essential for the expression of sexuality.

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SUMMARY

In mixtures of planospores derived from two or more epibiotic sporangia of *Physoderma pulposum*, several planospores function as gametes, pair, and fuse to form zygotes. Planospores from single epibiotic sporangia, or from germinating resting sporangia, however, do not copulate but infect the host as zoospores and give rise to epibiotic sporangia. The gametes form pairs, clumps, or long chains. After continuing together for more than 1 hour the pairs may separate, or ultimately settle down and fuse. In the later case, one of the gametes of the pair becomes sluggish and settles down, whereas the other gamete remains active for some time more. When both are quiescent, they develop pseudopodia and fuse. The resulting zygotes are feebly motile, and they may be uni- or biflagellate depending on whether or not the flagellum of one of the gametes was absorbed before plasmogamy. After a brief period of motility the zygote settles down and absorbs the flagellum. Karyogamy occurs at this stage.

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THE MYXOMYCETES OF THE MUSSOORIE HILLS (INDIA). X

G. W. Martin, K. S. Thind, and P. S. Rehill

(with 2 figures)

This paper deals with two new species of Myxomycetes collected from the Mussoorie hills in the northwest Himalayas (5000–7500 ft alt.). The first nine contributions (2–10) give accounts of 61 known species (including one variety), and three species and one form described as new. The two new species here described constitute nos. 66 and 67 of this series. The classification of Martin (1) has been followed throughout.

66. **Physarum retisporum** sp. nov. Fig. 1.

Fructificationibus plasmodiocarpis vel sporangiiformibus, lateraliter valide compressis, flavo-brunneis, rima tenui dehiscentibus; peridio duplici, membrano exteriore levi, granulis calcareis minutis aeque onustis; membrana interiore hyalina, iridescente, membranum exterius adpressente; capillitio copioso, nodis flavis, angulis; sporis obscure violaceo-brunneis, valde reticulatis, 9–11 μ diam.; plasmodio aureo.

Fructification plasmodiocarpous, often broken into sporangiate segments in more or less plasmodiocarpous arrangement, strongly compressed laterally, bright yellow-brown, dehiscent at outer margin; peridium double, the outer layer smooth, composed of densely compressed globose, yellow lime granules 1–2 μ in diameter, golden yellow within; the closely attached inner membrane transparent, iridescent, colorless except as colored by adhering granules of the outer layer, tending to separate at dehiscence; capillitium dense, the nodes large, yellow, angular, often massed in irregular transverse bands tending to separate the spore chamber into compartment-like sections, the nodes connected by pale yellow, noncalcareous tubules; spores black in mass, dark purplish brown under the lens, strongly reticulate, 9–11 μ in diameter; plasmodium bright yellow or yellowish orange.

India: Jamna Bridge, Mussoorie, 25 July 1954. *K. S. Thind 249*, type. In herb. Panjab Univ.; Iowa.

The *Diderma*-like peridium suggests that of *Leocarpus fragilis* but the shape, capillitium and spores are quite different. The massive capil-

litium suggests that of *Physarella oblonga* but trabeculae are lacking. The shape and color call to mind some forms of *Physarum bivalve* and *P. bogoriense*. From all these species it is distinguished by the reticulate spores. It is perhaps closest to *Physarum echinosporum*, which has been collected in the same area and has semi-reticulate spores, but is pure white in color. From that species it is distinguished not only by the color but by the strongly compressed fructification, the character

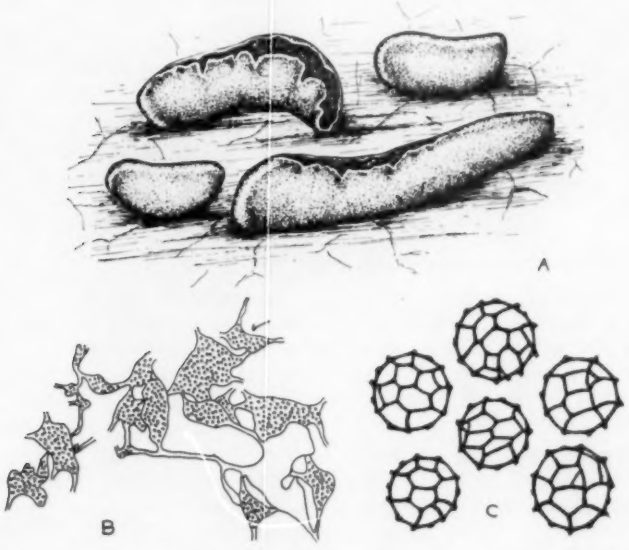

FIG. 1. *Physarum retisporum*. A. Group of sporangia, × 20. B. Capillitium, × 320. C. Spores, × 1150.

of the peridium and by the reticulation of the spores, which in *P. echinosporum* is clearly composed of lines of warts and is never complete. Also, the spores of *echinosporum* are somewhat larger, 11–13 μ, and much paler.

67. **Didymium floccosum** sp. nov. FIG. 2.

Fructificationibus stipitatis, gregariis, applanato-globosis, umbilicatis, 0.4–0.6 mm diam., 1.5 mm altis; peridiis floccosis, crystallis aggregatis tectis; columella clavata, nigra, crystallis calcareis repleta; floccis capillitii flexuosis, gracilibus, subfuscis, rare ramosis, in reticulum junctis; stipite stricto, externe levi, interne

crystallis calcareis repleto; sporis nigro-fuscis, verrucosis, 8–10 μ diam.; plasmodio ignoto.

Sporangia stalked, gregarious, flattened-globose, or globose, umbilicate below, 0.4–0.6 mm in diameter, up to 1.5 mm tall; peridium ashy, composed of a thin translucent membrane covered with clustered crystals, in dehiscence tending to break up into minute scales, each scale bearing a cluster of crystals; columella dark, clavate, rather small, not exceeding one-half of peridial cavity: capillitium dark, delicate, not exceeding 1.5 μ in diameter, sometimes bearing nodular thickenings, flexuous, sparingly

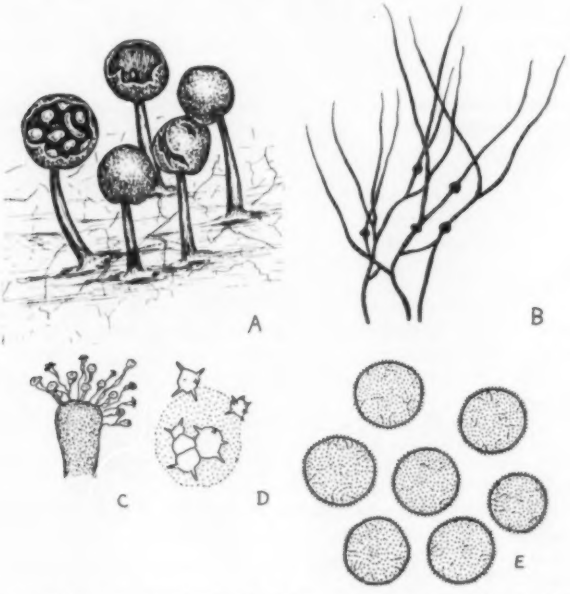

FIG. 2. *Didymium floccosum*. A. Group of sporangia, × 20. B. Capillitium, × 320. C. Columella with peridial scales attached to it by capillitial threads, × 50. D. Peridial scale, with crystals, × 500. E. Spores, × 1150.

branched and anastomosing; stalk ochraceous, darker below, smooth externally but packed with densely aggregated lime granules, up to 1.2 mm in height, 0.2–0.3 mm in diameter at base, tapering to 0.1 mm above, arising from an inconspicuous rotate hypothallus; spores black in mass, dark violaceous brown under lens, globose to broadly oval, densely verrucose, the warts often arranged in an obscurely reticulate pattern, 8–10 μ in diameter.

India: Forest Research Station, Dehra Dun, 22 August 1953, *K. S. Thind 250*, TYPE. In herb. Panjab Univ.; Iowa.

The dark ochraceous, calcareous stalks, the rather small, very dark, strongly verrucose spores with suggestion of a partial reticulation due to the lines of closely spaced warts, and the arrangement of the crystals on the peridium in small clusters tending to break away as small flakes in dehiscence do not fit any previously described species of *Didymium*. The crystals are composed of a subglobose central portion from which the long spines diverge.

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NEW SPECIES OF MYXOBACTERIA FROM THE BARK OF LIVING TREES[1]

JOHN E. PETERSON

(WITH 8 FIGURES)

The myxobacteria have been known predominately from soil and soil-like habitats, such as dung, compost and decaying plant materials. The bark of living trees has not been investigated either as a habitat or as an isolation substrate for myxobacteria. It is readily understandable, therefore, that critical examination of the bark of living trees for species of the order Myxobacterales (Schizomycetes) should result in the finding of previously undescribed species of this group of organisms. This paper describes four such species.

Stelangium vitreum sp. nov.

(Etymology: Latin *vitreum*, glassy.)

Fruiting bodies delicate crystalline orange in color, sessile on substrate, simple, either globose or columnar, generally unbranched, 60–70 μ in diameter, ranging up to 150–200 μ for the more complex, columnar forms. In transmitted light, no membrane or wall discernible; encysted rods loosely parallel in packets, 6–12 per packet; adjacent packets irregularly perpendicular to each other in basket-weave fashion, this effect best observed under high magnifications. Encysted rods straight with squarish ends, uniform, $0.7 \times 2.0\ \mu$, staining unequally with aniline blue dye to appear darker at ends and in middle, these areas appearing to bulge. Vegetative cells $0.7 \times 4.0\ \mu$.

Type material: HOLOTYPE *Peterson 62*, on bark of *Quercus velutina* growing in Sinkin Forest, Dent County, Missouri, Moist-chamber Culture No. 241-16, in the University of Missouri herbarium. Paratypes, *Peterson 64*, on bark of *Juglans nigra* growing in Ashland Wildlife Area, Ashland, Missouri, Moist-chamber Culture No. 202-d-2-2, in the herbarium of the State University of Iowa; and *Peterson 63*, on bark of *Juniperus virginiana* growing at Providence, Missouri, Moist-chamber Culture No. 210-g-12, in the Farlow Herbarium, Harvard University.

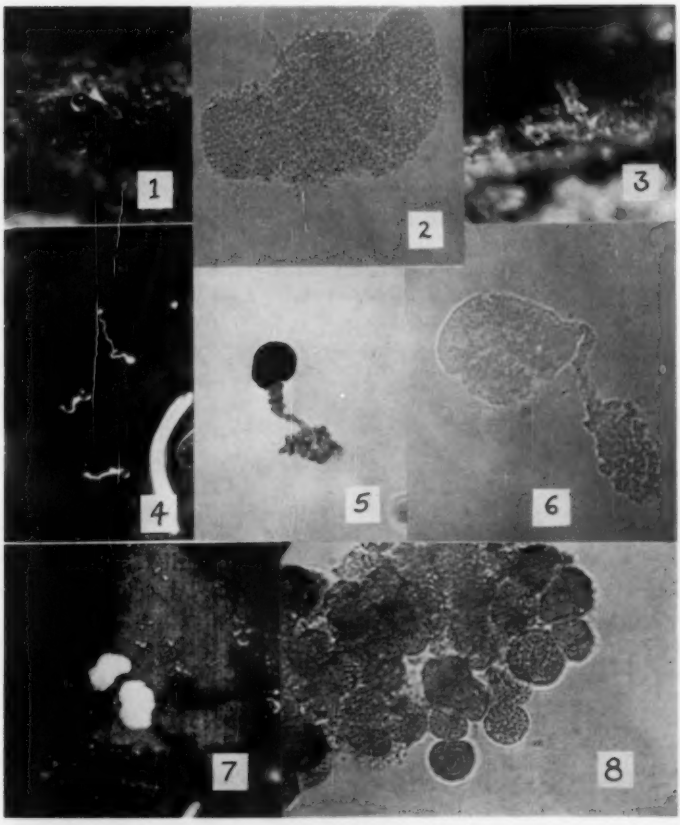
[1] A portion of a thesis presented to the School of Advanced Graduate Studies of Michigan State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Recorded collections: Found on 15 different pieces of bark from 10 different trees, representing six different species in six different geographic locations in Missouri. Bark collected from the following trees and locations: *Juglans nigra,* Ashland Wildlife Area, Ashland, Missouri; *Quercus alba,* Ashland Wildlife Area, Ashland, Missouri; *Juniperus virginiana,* Providence, Missouri; *Ulmus americana,* the Pinnacles, Boone County, Missouri; *Quercus alba,* Sandstone Bluffs, Boone County, Missouri; *Carya ovata,* Ashland Wildlife Area, Ashland, Missouri; *Quercus velutina,* Sinkin Forest, Dent County, Missouri; *Carya ovata,* Wagner's Woods, Holstein, Missouri; *Quercus alba* (2 collections), Wagner's Woods, Holstein, Missouri.

The genus *Stelangium* was erected by Jahn (**1915**) to encompass only one species, Thaxter's *Chondromyces muscorum* (**1904**). This organism, now *Stelangium muscorum* (Thaxter) Jahn, is the closest relative to *S. vitreum. S. muscorum* had not been reported since Thaxter's original description in 1904 until the writer found it on the bark of living trees. The writer has examined Thaxter's type material, now on deposit in the Farlow Herbarium, in addition to the specimens of *S. muscorum* found on bark of living trees. *S. vitreum* differs markedly from *S. muscorum* in the persistent crystalline, almost transparent, quality of the fruiting bodies. *S. muscorum* fruiting bodies are essentially opaque in character. The specific epithet was selected to describe this beautifully glistening characteristic of the fruiting bodies of *S. vitreum* (FIG. 1). The encysted rods of *S. vitreum* are smaller than those of *S. muscorum;* they exhibit a "three-beaded" appearance when stained whereas those of *S. muscorum* do not; and they are arranged in indistinct basket-weave packets, while those of *S. muscorum* give no suggestion of any such arrangement.

The fruiting body of *S. vitreum* is pale pink in color in transmitted light when first mounted. Lactophenol soon bleaches this color, however, and the mass becomes hyaline in appearance. In cotton-blue-stained preparations there is a layer of amorphous material which takes stain and separates off from the encysted rod mass proper to produce an over-all illusion of a halo around the spore mass. Some of this material appears to be globules of an oily substance. This material is interpreted to be the slime envelope, or residue of the slime envelope, around the spore mass, even though a slime envelope as such is never discernible in the intact fruiting body (FIG. 2).

Attempts to culture *S. vitreum* have not been completely successful, and it has not been grown on an agar medium. Fruiting bodies have been produced on sterilized elm bark two weeks after the bark was



FIGS. 1–2, *Stelangium vitreum;* FIG. 1, habit, × 90; FIG. 2, whole mount, × 570. FIG. 3, *Podangium cylindricum,* habit, × 90. FIGS. 4–6, *Podangium alboracemum;* FIG. 4, habit, × 90; FIG. 5, whole mount, stained, × 240; FIG. 6, whole mount, showing spores, × 570. FIGS. 7–8, *Angiococcus moliroseus;* FIG. 7, habit, × 45; FIG. 8, whole mount, stained, × 1080.

seeded with cysts. Elm bark is an extremely difficult substrate on which to observe colony characteristics, but small, yellowish-gray slimy areas were found on such bark preparations previous to the formation of fruiting bodies. Mounted portions of these slimy areas showed definite myxobacterial vegetative cells in combination with true bacterial cells. There is little doubt that these vegetative cells belong to *S.*

vitreum, but more critical study of the vegetative phase in pure culture is certainly a necessity for the future.

Podangium cylindricum sp. nov.

(Etymology: Greek *kylindrikos*, cylindrical.)

Fruiting body consists of a cylindrical cyst on a short cystophore, many arising from a common hypothallus-like base to produce an aggregate of fruiting bodies; aggregates up to 350 μ in size and containing up to 70–100 fruiting bodies. Cysts brownish-orange, up to 40 × 105 μ, rounded at apex, square at base. Cystophores whitish at base, grading to yellow at apex, irregular in size, usually $\frac{1}{4}$ to $\frac{1}{3}$ as long as the cyst and $\frac{1}{2}$ as wide, composed completely of rods in dried slime. Cyst contains oily globules and spores; oily globules sometimes large and prominent, sometimes very small. Encysted rods 0.8 × 2.5–3.5 μ, slightly curved, tapering toward both ends, mostly crescent-shaped. Vegetative cells 3.5–5.5 μ long, occasionally up to 7.0 μ, 1.0 μ in width in center of cell, tapering toward both ends, straight. Vegetative colony yellowish in center, pink at periphery.

Type material: HOLOTYPE *Peterson 70*, on bark from living *Cupressus sempervirens* growing at Olympia, Elis, Greece, collected and cultured in moist chamber by *C. J. Alexopoulos* on April 15, 1955, Moist-chamber Culture No. 180, in the University of Missouri herbarium. Isotype bearing *Peterson no. 71*, in the Farlow Herbarium, Harvard University. Paratype *Peterson 38*, with all data the same as for the holotype except that it bears Moist-chamber Culture No. GR-231, in the herbarium of the State University of Iowa.

Recorded collections: *Podangium cylindricum* is known only on two different pieces of bark collected from a living *Cupressus sempervirens* growing at Olympia, Elis, Greece. Several hundred fruiting bodies were scattered over one of these pieces of bark (*Alexopoulos* Moist-chamber No. *180*) and this piece of bark was divided to make the holotype collection and the isotype collection. The second piece of bark (Alexopoulos Moist-chamber No. GR-231) also had many fruiting bodies on it; this collection is the above-described paratype.

Although the fruiting bodies of *P. cylindricum* were a bit over a year old when they came into the hands of the writer, profuse culture, in almost initially pure colonies, was readily accomplished. Vegetative colonies and some fruiting bodies were produced four days after seeding cysts on sterilized rabbit-dung pellets and on bark-decoction agar. Vegetative growth without the production of fruiting bodies occurred on rabbit-dung-decoction agar and on a medium made of agar and auto-

claved eubacterial cells. Vegetative growth was extremely good on rabbit-decoction agar, but no fruiting bodies were formed on this medium. On the other hand, excellent and rapid fruiting occurred on sterilized rabbit-dung pellets in water agar, but vegetative growth was at a minimum. The other two media named were both very inferior in supporting either vegetative growth or fruiting. Two weeks after seeding cysts on autoclaved elm bark, many aggregates of fruiting bodies were produced on the bark in conjuction with heavy blankets of fungus contamination.

Podangium cylindricum has very definite similarities to *Podangium erectum* (Schroeter) Jahn (**1924**). The writer has strong tendencies toward being a "lumper," but he is convinced that the two are different organisms and that he can readily tell them apart. The writer has recorded *P. erectum* on bark from living trees fifteen times. In addition, through the courtesy of Dr. I. Mackenzie Lamb, he has examined three of Thaxter's collections of *P. erectum* now on deposit in the Farlow Herbarium. Finally, he has succeeded in growing both species in culture (not necessarily pure culture, however) and in ascertaining that the differences between the two are constant.

The reasons for the conclusion that *P. cylindricum* is a distinct species from *P. erectum* are: (1) The size and shape of the cysts of the two organisms differ, those of *P. cylindricum* being longer and typically cylindrical, as the assigned specific epithet indicates; (2) the cystophores of *P. cylindricum* are composed of rods, whereas those of *P. erectum* are composed of slime without prominent rod inclusion; (3) the oily globules in the cyst contents of *P. cylindricum* have not been reported in the literature for *P. erectum* and could not be found in any of the specimens of *P. erectum* examined; and, (4) both the spores and the vegetative cells of *P. cylindricum* are somewhat larger than those of *P. erectum*, although they are much like their respective counterparts except for this slight, possibly insignificant, difference in size.

Podangium alboracemum sp. nov.

(Etymology: Latin *albus*, white; Latin *racemus*, stalk.)

Fruiting body a single cyst on a long, white, irregularly corkscrew-shaped cystophore; 85–250 μ (125 μ average) tall; deliquescent. Cysts irregular globes, pale orange, crystalline, about 35 μ in diameter, bounded by an elastic, indiscernible membrane when fresh. Cystophore 4–6 times as long as cyst diameter, 20 μ wide, snowy-white, sharply angled and irregularly corkscrewed, composed of amorphous material. Encysted rods 0.8 × 2.5 μ, slightly curved, difficult to separate from cyst and from

each other. Vegetative cells 0.8–1.0 × 4.5–5.0 μ; no tapering apparent; square ends.

TYPE material: Three microscope slides, *Peterson 72,* Moist-chamber Culture No. 282-3, in the University of Missouri herbarium.

Recorded collections: On bark of *Ulmus americana* collected from a living tree growing at Round Spring, Missouri, in May, 1956; consisting of about three dozen fruiting bodies. On a second piece of bark collected in March, 1956, from a living *Ulmus americana* growing near Perryville, Missouri; consisting of about one dozen fruiting bodies. Both trees were growing in moist, wooded river-bottom habitats. The Round Spring, Missouri, collection is the one from which the type specimens were mounted and this is, therefore, the type locality.

Podangium alboracemum is perhaps the most distinctive, most striking and most interesting of the more than 60 species of myxobacteria that the writer has studied. It is obviously a member of the genus *Podangium,* but it cannot possibly be confused with any of the other members of the genus. When this organism was first found, some notations were made and the piece of bark was set aside for further study at a later date. Some time later, when the second occurrence presented itself in a moist, formative state, the first piece of bark was again examined. Absolutely no trace of the fruiting bodies originally seen on the first piece of bark could be found, although every millimeter of the bark was examined under 90 × magnification. The first piece of bark was rewet in an effort to get the organism to complete its life cycle again, while mounts, photographs and critical notations were made of those fruiting bodies of the second occurrence. Vegetative cells were seen in mounts made from material taken directly from the second piece of bark as such material was rising into fruiting bodies. Suddenly and unexpectedly, within a period of 24 hours, the cysts on the second piece of bark also deliquesced and disappeared. Both pieces of bark were rewet twice, but the fruiting bodies of *P. alboracemum* have never again been seen.

In consequence, neither specimens in situ nor cultures of *P. alboracemum* are in existence. However, excellent photographs and microscope slides are evidence of the existence of this will-o'-the-wisp organism. This explains why microscope slides have had to be designated as type material. The deliquescent nature of this species is particularly interesting in view of the facts that it has never been reported for any other of the myxobacteria and that the writer has never observed it, even in moist-chamber culture, for any of the other myxobacterial species.

The fruiting bodies of *P. alboracemum* consist of a single cyst on

the end of a long, snowy-white, irregularly corkscrewed cystophore. The structure presents a very ungainly appearance because the length of the cystophore is from four to six times are diameter of the cyst. The fruiting bodies are small, averaging about 125 μ in total height, and are barely discernible at 15 × magnification under the stereoscopic microscope.

The cysts are irregular globes, pale orange and crystalline in reflected light. In transmitted light, they appear hyaline with only a trace of orange or yellow color. They are bounded by a wall which is distinctly gelatinous and elastic and quite indiscernible, but tough enough to hold the cyst together during rather harsh treatment. The spores within the cyst are clearly visible and show no particular orientation.

The cystophores of *P. alboracemum* are the outstanding characteristic, and they suggested the specific epithet that has been assigned to the organism. They are pure snowy-white in reflected light, but completely hyaline in transmitted light. Before mounting, they are sharply angled in irregular-corkscrew fashion in a striking, but undescribable, manner (FIG. 4). The cystophores are composed of amorphous material which is very uniform in appearance and in which no vegetative cells or other structures are found. This material has very little affinity for aniline-blue dye and the cystophore stains only faintly.

Angiococcus moliroseus sp. nov.

(Etymology: Latin *moles,* mass; Latin *roseum,* rosy.)

Fruiting bodies irregularly shaped pink cushions of tiny cysts, resembling a pile of pink sand grains; less than 100 μ in size, 50–60 μ high. Cysts pink in mass, hyaline individually, 6–15 μ in diameter, averaging 10 μ. Cyst wall thin, tough, smooth, often with black, refractile granules on surface. No discernible connection between cysts; no discernible slime holding cysts together. Encysted rods (spores) of two types: one type spherical, measuring 0.8–1.0 μ in diameter, difficult to free from cyst, staining avidly with aniline blue; other type spherical (1.2 μ in diameter) to shortened rods (1.0 × 1.5 μ) with gradients between, the predominant form being oval, 0.8 × 1.3 μ, somewhat separable from cyst, with no avidity for aniline blue. Vegetative cells not described.

Type material: HOLOTYPE *Peterson 30,* on bark from a living *Ulmus americana* growing on the west edge of Tucker Prairie, Missouri, Moist-chamber Culture No. 1B-17, in the herbarium of the University of Missouri. Paratypes, bearing *Peterson 9,* other data as for the holotype except that the Moist-chamber Culture No. is 3B-17, in the Farlow Herbarium; *Peterson 18,* other data as for the holotype except that the

Moist-chamber Culture No. is 3B-7, in the herbarium of the State University of Iowa; and *Peterson 8*, other data as for the holotype except that the Moist-chamber Culture No. is 2B-14, in the University of Missouri herbarium.

Recorded collections: In addition to the four records described above, each of which consisted of some two to three dozen fruiting bodies and all of which were collected in June, 1957, *Angiococcus moliroseus* has been found on one other piece of bark from a living tree. This bark was again from an *Ulmus americana*. The tree was growing in an open, dry situation at the Pinnacles, Boone County, Missouri, and the bark was collected in April, 1955. The collection consisted of about 40 fruiting bodies.

The fruiting bodies of *A. moliroseus* are irregularly shaped piles of very tiny cysts. The shape of the pile is predominately a cushion, but it may be irregularly elongate and not necessarily smooth on the surface. In fact, the fruiting body may be described as appearing to be a pile of adhering sand grains. In reflected light, the fruiting bodies are a lovely, delicate pink in color and the specific epithet assigned to the organism was selected to describe this "pink mass." There is no suggestion of a stipe or cystophore and the mass simply lies on the substrate, which, in most cases, is bare bark. The fruiting bodies are extremely variable in shape and size.

In transmitted light the cysts are faintly pink in mass; observed individually, they are quite hyaline. Many of the cysts are covered with masses of tiny, black, refractile granules, which are almost the same size as the spores inside the cysts, often to the extent of making the entire cyst appear black. The black granules disappear when heated in lactophenol, leaving the cysts clear and hyaline. Such heating also destroys some of the faint pink coloration. The granules appear to be trapped in the dried slime composing the cyst wall, but no opinion is presented as to what they are, whether they actually belong to the myxobacterial organism, or whether they are extraneous material.

The spores of *A. moliroseus* are not completely understood at this writing. All of them are difficult to free from the cyst walls and when this is accomplished the spores still adhere in irregular masses. It is therefore difficult to find individuals for study and measurement. However, there appear to be two different types of spores present in the cysts. One type is essentially spherical in shape, with some irregularities due to pressure in mass, and measures about 0.8–1.0 μ in diameter. These take aniline blue well and are extremely difficult to remove from the cyst. The second type ranges from spherical to shortened rods in

shape with the majority of them being oval and measuring $0.8 \times 1.3\ \mu$. The more spherical spores of this type measure $1.2\ \mu$ in diameter and the rod-shaped ones measure about $1.0 \times 1.5\ \mu$ in size. There are, of course, gradients between these extremes, with the oval-shaped spores representing the mean. These various spores of the second type show a lack of affinity for aniline blue and appear hyaline in stained preparations. They are also somewhat easier to separate from the cyst wall than are the first type.

Attempts to culture *A. moliroseus* on five different media were unsuccessful. Nothing is known of the vegetative phase of the organism nor of the development of the fruiting body at this writing.

The genus *Angiococcus* Jahn contains two other species in addition to the one now being added. Neither of these two species has been seen by the writer and, consequently, they are known to him only by descriptions and sketches. The first of these, *A. disciformis*, described by Thaxter (**1904**) and later reported by the Krzemieniewskys (**1926**), produces fruiting bodies composed of orange, disc-shaped cysts and does not appear to have any close affinities to *A. moliroseus*. The second species of the genus, *A. cellulosum* Mishustin (**1938**), does have some characteristics in common with *A. moliroseus*. *A. cellulosum* produces yellow or pink, rounded fruiting bodies. This description also could fit that for *A. moliroseus*. The cysts of both species appear to be of the same general size and shape. Mishustin has described the spores of *A. cellulosum* as being shortened rods in globular masses that are easily broken up, but he has indicated no size for the spores. The description of "shortened rods" could easily enough fit some of the spores found in *A. moliroseus*, but the spores of *A moliroseus* are anything but easily broken up. The cysts of *A. cellulosum* are surrounded by an outer wall or envelope which is supported on from one to several short stalks or cystophores. No such structures are found in *A. moliroseus* and this is the prime difference—and a substantial one—between the two species in question.

SUMMARY

Examination of the bark of living trees for representatives of the order Myxobacterales (Schizomycetes) has resulted in the finding of several previously undescribed species. Four such species of myxobacteria, *Stelangium vitreum*, *Podangium cylindricum*, *Podangium alboracemum*, and *Angiococcus moliroseus*, are described.

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THE GENUS ZYGORHYNCHUS

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(with 19 figures)

The genus *Zygorhynchus* was established in 1903 by Vuillemin, who classified the known genera of Mucorales on the basis of zygospore apparatus and placed the genus in his group Zygorhynchées, which was characterized by the formation of zygospores by the union of two unequal and dissimilar progametangia. The genus *Zygorhynchus* was distinguished by Vuillemin from the only other genus in his group, namely *Dicranophora*, by the progressive inflation of the larger suspensor in the former as opposed to the abrupt inflation in the latter genus and by the zygospore apparatus's being borne from aerial hyphae rather than from the substrate mycelia. Since 1903 all students of the Mucorales, except Lendner (**1908**), have recognized this genus. Its heterogamic nature has always been considered the principal character separating this genus from *Mucor*. Lendner ignored this character and included the two species of *Zygorhynchus* then known, *Z. moelleri* Vuill. and *Z. heterogamus* Vuill., as species of the genus *Mucor*.

Since 1903 seventeen new species and varieties of *Zygorhynchus* have been described. In 1939 Naumov listed thirteen species of *Zygorhynchus*, including one new species of his own, viz. *Z. hennebergii* Naumov. Some of these species had significant differences; others were separated on variable characters of no taxonomic value. The principal characters distinguishing the species were: (1) colony color, (2) size and shape of spores, (3) size range of sporangia, and (4) size range of zygospores. In many cases the difference in size range of various structures was so insignificant that it was hard to distinguish one species from another. Realizing the ambiguity of the situation, Zycha (**1935**) in his monograph of the Mucorales recognized only six species in the genus. Since publication of this monograph one new species of the genus, viz. *Z. verruculosus* Tehon, has been described (Tehon, **1943**).

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Students of the Mucorales have from time to time noted the need for a revision of the species concept in this genus (Jensen, **1912**, Tehon, **1943**, Hesseltine, **1950**). One of the main reasons for ambiguity among the species of the genus *Zygorhynchus* has been the lack of detailed descriptions of the earlier known species based primarily on a complete study of morphological characteristics of the species on reproducible media. The present study was, therefore, undertaken to re-examine all the available strains of the different species of *Zygorhynchus*. Our taxonomic descriptions are based on observations of morphology and growth habits as seen on synthetic mucor agar (SMA) (Hesseltine, **1954**) and on potato-dextrose agar (PDA) (Hesseltine, **1950**). Whenever necessary malt-extract agar and Czapek's-solution agar (Haynes et al., **1955**) were also used.

Zygorhynchus is very closely related to *Mucor*. It produces the typical spherical columellate *Mucor*-like sporangia and forms branched sporangiophores that bear sporangia containing many sporangiospores. *Zygorhynchus* differs from *Mucor*, however, in that all species are homothallic, and the zygospores are borne between suspensors of unequal size and shape. *Mucor* is typically heterothallic. In a few species of *Mucor* the homothallic condition occurs, but in these the suspensors are almost equal and are usually produced on special zygophores near the surface of the substrate and not throughout the aerial portion of the colony as they are in the genus *Zygorhynchus*.

The zygospores of *Zygorhynchus* are typically formed on branches of sporangiophores—occasionally on other hyphae. Near the ends of the hyphae septa form, delimiting short, straight, terminal portions which from their sides put forth short, straight progametangia at right angles to the hyphae. At maturity, these suspensors remain short and uninflated. From directly below the septum the second progametangium arises as a lateral outgrowth; it is longer than the other progametangium and recurves upward to come in contact with its mate. Delimitation of the gametangia leaves two very unequal suspensors between which the zygospore is formed.

GENERIC DESCRIPTION

ZYGORHYNCHUS Vuillemin, Bull. Soc. Mycol. France **19**: 116. 1903.

Colonies white to dark gray (at times orange, especially at 32° C), arachnoid or cottony, with mycelium immersed, aerial or prostrate; aerial mycelium variable in amount, mostly nonseptate, branched; chlamydospores smooth, intercalary or terminal, differing in frequency

from strain to strain and from medium to medium; sporangiophores isolated or in groups, irregularly branched, bearing both zygospores and sporangia; sporangia globose, many-spored, deliquescing or breaking, without apophyses, with variously shaped columellae; sporangial walls sometimes encrusted, leaving collars; sporangiospores small, smooth to slightly roughened, variously shaped, colorless or occasionally grayish in mass; progametangia very unequal, typically produced at the ends of sporangiophore branches, less commonly at the ends of other hyphae, the smaller progametangium and the terminal hyphae cut off from the hyphae by a septum, the larger formed as a lateral outgrowth just below the hyphal tip; zygospores always present, globose to somewhat flattened, heavy-walled, brownish or black, covered with projections; suspensors unequal in size and dissimilar in shape, with the smaller one short and straight and the larger one longer, curved and terminally expanded; homothallic.

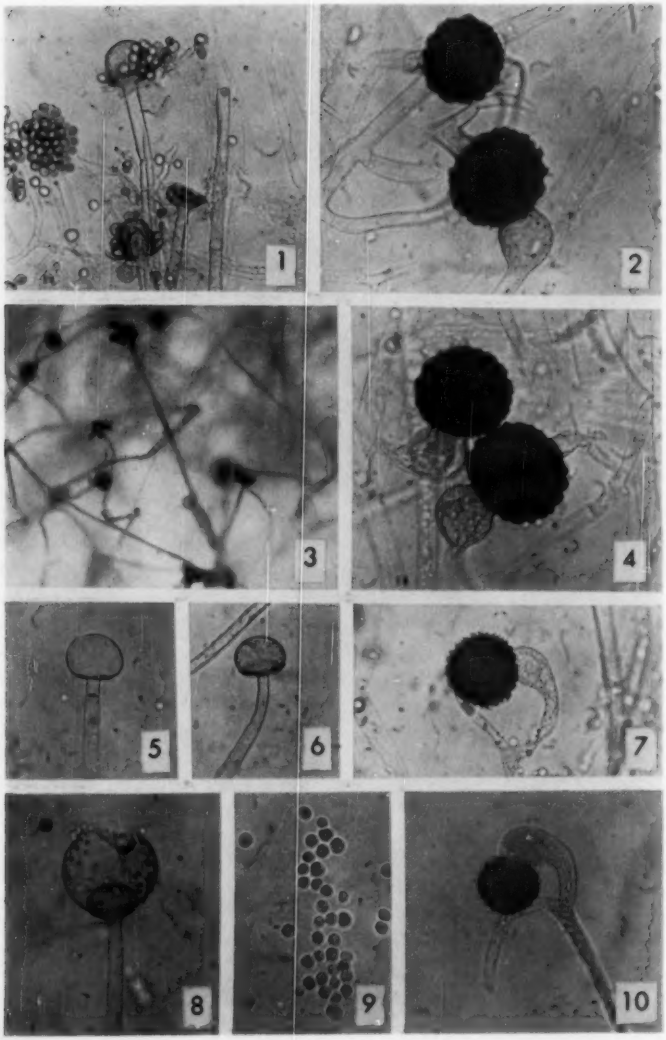
TYPE species: *Z. heterogamus* Vuillemin.

This genus has been reported exclusively from soil and is world-wide in distribution. The species are found especially in sandy subsoils poor in organic matter (Waksman, **1932**). According to Hagem (Waksman, **1932**), *Zygorhynchus* may form mycorrhiza with forest trees. It is also said to be active in forming ammonia from protein sources such as dried blood and cottonseed meal (Waksman, **1932**).

In our studies on carbon assimilation, all species of *Zygorhynchus* have been found to utilize L-xylose, glucose and glycerol, but not lactose, starch or cellulose as carbon sources. From among 11 strains tested only 3, NRRL Nos. 1497, 1494 and 2658, utilized sucrose.

KEY TO THE GENUS ZYGORHYNCHUS

1. Sporangial wall splitting open from the dorsal side exposing the sporangiospores in undisturbed cultures; sporangiospores rough and always globose 2
   2. Sporangia dark gray in reflected light ..... 1. *Z. exponens*
   2. Sporangia tan or yellow in reflected light ..... 2. *Z. exponens* var. *smithii*
1. Sporangial wall not splitting open in undisturbed sporangia; sporangiospores smooth and variously shaped 3
   3. Larger suspensor always abruptly enlarging and hence this enlargement globose to pyriform in shape; large mature zygospores black 5
   3. Larger suspensors gradually enlarging towards the zygospore; mature zygospores brown or even dark brown but seldom black 4
4. Sporangiospores regularly spherical or globose ..... 4. *Z. californiensis*
4. Sporangiospores oval, oblong or nearly elliptical in shape 6
   5. Sporangiospores oval; sporangia 50–100 μ in diam ..... 6. *Z. macrocarpus*
   5. Sporangiospores oval to globose; sporangia never more than 60 μ in diam ..... 5. *Z. heterogamus*



FIGS. 1–3. *Zygorhynchus exponens.* FIG. 1. Sporangiophores, showing columellae and sporangiospores. FIG. 2. Zygospores. FIG. 3. Sporangia opening in place. FIGS. 4–5. *Z. exponens* var. *smithii.* FIG. 4. Zygospores. FIG. 5. Sporan-

6. Columellae pyriform to globose; sporangiospores to 10 μ in length.....
.....*7. Z. japonicus*
6. Columellae applanate; sporangiospores not more than 7 μ in length.....
.....*3. Z. moelleri*

1. ZYGORHYNCHUS EXPONENS Burgeff, Bot. Abhandl. **4**: 34. *figs. 11–14.* 1924. FIGS. 1–3

Zygorhynchus polygonosporus Pispek, Acta Bot. Inst. Univ. Zagrebensis **4**: 93. *fig. 8.* 1929.

Colonies from 1–4 mm in height, not covering media of Petri dishes in 8 days, Pale Olive-Gray [2] to Pallid Mouse Gray, after 1 month Light Grayish Olive; colony reverse Pale Olive-Buff, becoming Dark Olive-Buff at 1 month; odor sweet but disappearing with age; margin of colonies irregular from sporangiophores' touching medium and growing; sporangiophores arising from substrate mycelium, usually erect, hyaline, often septate especially just above origin of branch, smooth but often roughened below sporangium and with roughenings much more pronounced in age, up to 20 μ in diam., not phototrophic, sympodially branched; sporangia at first white but at maturity dark gray, borne erect, always breaking or splitting open even when undisturbed, globose to slightly applanate, many-spored, up to 70 μ (occasionally to 90 μ) in diam.; sporangial wall hyaline, smooth, transparent; columellae applanate to globose (conical ones have been seen on Czapek's-solution agar), light gray, with collar, smooth-walled, 6–26 × 10–42 μ; sporangiospores very variable in shape, globose, short-oval, irregular with U-shapes (on Czapek's-solution agar mostly globose with some larger ones oval and with wall roughened, globose spores 3.5–6 μ with few larger oval spores 7.5 × 8.5 μ), smooth to faintly roughened, light gray, with contents granular or vacuolate, where globose 4.5–8.5 μ, where atypical to 9 × 17 μ; chlamydospores in sporangiophores usually solitary, intercalary, cylindrical, smooth-walled, with uniform contents, up to 15 × 26 μ, seen best in old cultures; zygospores abundant near surface of medium, reddish brown to dark brown, globose to slightly compressed, with blunt projections up to 6.5 μ in length, usually surrounded by a droplet of liquid when seen in place, 32–80 μ in diam.; zygophores branched, hyaline, roughened, up to 9 μ in diam.; suspensors unequal (some nearly the same size), light brown in color, slightly roughened, 8–26 μ in diam. for the smaller suspensor, 20–36 μ in diam. for the larger; homothallic.

[2] All colors that are capitalized are those used by Ridgway (**1912**).

giophore with columella. FIGS. 6–7. *Z. moelleri.* FIG. 6. Sporangiophore with columella. FIG. 7. Zygospore. FIGS. 8–10. *Z. californiensis.* FIG. 8. Sporangiophore with ruptured sporangium and columella. FIG. 9. Sporangiospores. FIG. 10. Zygospore.

TYPE strain: NRRL 1492, the strain used by Burgeff to describe the species. The above description is based on the type strain grown on SMA for 8 days at 25°.

This species has been reported only from Europe, having been found by Burgeff in forest soil at Geisenheim, by Pispek in soil in Jugoslavia, and by Zycha in soil near Barcelona.

Colonies on Czapek's-solution agar appear thin and colorless with many sporangiophores but no zygospores. Zygospores are produced on malt and PDA, and on each of these media the colonies are over 0.5 cm in height.

The manner of formation of zygospores is definitely intermediate to those found in *Z. moelleri* and the homothallic *Mucors*. Whether the suspensors are markedly different in size and shape or approximately identical depends upon the conditions under which the culture is grown. For example, on corn-steep-liquor agar the suspensors are nearly equal and often arise from different zygophores. On the other hand, on hay agar the suspensors are unequal and some are characteristically *Zygorhynchus*-like in that one suspensor is large, long and bent back upon itself. On other media, such as yeast-extract and malt-extract agars, both the *Mucor* condition and the *Zygorhynchus* condition exist. This species is being retained in *Zygorhynchus* for the following reasons: on some media the typical *Zygorhynchus* manner of formation of zygospores is present; earlier investigators have always retained it in this genus; its pattern of utilization of carbohydrates is similar to that of other species of *Zygorhynchus;* and the presence of applanate columellae, its occurrence in soil, etc., are all typical of species of *Zygorhynchus*.

The sporangiospores as seen on SMA are obviously very abnormal. When seen on a medium such as Czapek's-solution agar, they are rather uniformly globose in shape and distinctly roughened. This species is distinct from other known species of *Zygorhynchus* because of its roughened globose spores which are especially evident on such a medium as Czapek's-solution agar, and because of the dehiscence of the sporangial wall in undisturbed sporangia.

Burgeff very briefly described *Z. exponens*, pointing out that sporangia were 54–108 μ in diameter, with sporangiospores globose, 3.5–7 μ in diameter and zygospores 54–81 μ in size with the spines on the zygospores 5.5–8 μ in length. His illustration of the sporangium is very informative, since it shows the sporangial wall splitting downward to expose the spores.

Pispek (**1929**) described this species again as *Z. polygonosporus*, describing the sporangiospores as 3.5–5.25 μ in diameter. The sporangia

measured 45–70 μ with zygospores 56–85.5 μ in diameter. According to Pispek the species is distinct from other species of *Zygorhynchus* because the sporangiospores are angled and the columellae are pyramidal and echinulate. Our view regarding the species *Z. polygonosporus* is in conformity with that of Zycha, who considered it a synonym of *Z. exponens*. Certainly Pispek's measurements are in complete agreement with ours. Interestingly, Pispek makes no mention of Burgeff's paper and was probably unaware of its existence. The spores found in this species, because of their roughenings, sometimes do appear to be angled. The appearance of the zygospores and their arrangement on the zygophores agree with the illustrations of Burgeff and Pispek.

2. ZYGORHYNCHUS EXPONENS var. **smithii** var. nov. FIGS. 4–5

Coloniae pallide murinae, in media leniter brunnescentes; sporangia flavidula usque in luce reflecta alutacea, 20–80 μ in diam.; columellae dorsiventraliter applanatae, usque ad 26 × 35 μ; sporangiosporae echinulatae, sphaericae, 4.5–6.5 μ; zygosporae globosae, rufo-brunneae usque atro-brunneae, episporio processibus 2–5 μ longis, 30–62 μ in diam., ruguloso. E solo isolatus, Anglia.[3]

Colonies from 8–9 mm in height, rather restricted (4 cm in diam. after 7 days), Light Mouse Gray, after 1 month Mouse Gray with a slight yellow-brown pigment in medium; colony reverse Deep Olive-Buff to Buffy Brown, after 1 month Buffy Brown to Olive-Brown; odor faintly sweet; sporangiophores arising from substrate, hyaline, smooth to very slightly roughened, roughened especially below sporangium, erect, sympodially branched, not phototropic, up to 20 μ in diam.; sporangia at first white then light yellow to tan in reflected light, borne erect, globose, sporangial wall breaking in place leaving many spores adhering to sporangial wall, 20–80 μ in diam.; columellae applanate (ovate in larger ones on PDA), hyaline to light brown, with collars not clearly seen, up to 26 × 35 μ (on PDA 20–52 × 20–52 μ); sporangiospores spherical, verrucose, with short spines giving an angled spore shape, with contents homogeneous, 4.5–6.5 μ; chlamydospores not seen: zygophores irregularly branched, potentially sporangiophores, bearing one or more zygospores, roughened, hyaline, up to 10 μ in diam.; zygospores mostly borne just above surface of substrate, globose, reddish-brown to brownish-black, surrounded by large conspicuous yellow-colored droplets, covered with blunt projections which are 2–5 μ in length, 30–62 μ in diam., average about 52 μ; suspensors usually unlike in size, the larger one growing out and bending back to the zygospore, in others nearly equal; smaller suspensor hyaline, or slightly colored, straight, smooth, enlarging only slightly next to the zygospore, often with a

[3] Latin description prepared by Miss Edith Cash.

septum a slight distance from the zygospore; larger suspensor roughened but nearly smooth next to the zygospore, colored in the swollen region, sometimes constricted next to the zygospore, with contents granular, 13–32 μ in diam.; homothallic.

The above description is based upon studies of NRRL 2572, the TYPE strain, grown on SMA at 25° for 7 days and observed over 1 month. Both sporangia and zygospores began to form in 48 hours. This variety is named in honor of Mr. George Smith, London School of Hygiene and Tropical Medicine, who submitted a culture of this fungus to the authors for study. This strain was sent to us as his *BB345*, isolated in 1956 from soil from a ploughed field, Monsall Dale, Derbyshire, England.

This variety is obviously related to *Z. exponens* because of its roughened, globose sporangiospores, its sporangial wall which dehisces in place, and the similarity in the formation of the zygospores. However, it is distinct from NRRL 1492, *Z. exponens*, by having slightly smaller sporangiospores and the columellae sometimes colored light brown, not grayish. It possesses sporangia which are light yellow or tan in reflected light.

This variety grows well on malt agar and PDA, forming both zygospores and sporangia. Upon malt agar the reverse side of the colony is wavy zonate as seen in *Mortierella*. Growth on Czapek's-solution agar is thin, but both sporangia and zygospores are produced.

3. ZYGORHYNCHUS MOELLERI Vuillemin, Bull. Soc. Mycol. France **19**: 117. 1903. FIGS. 6–7

Mucor moelleri (Vuill.) Lendner, Les Mucorinées de la Suisse. Matér. Flore Crypt. Suisse **3**(1): 72. *fig. 25.* 1908.

Zygorhynchus vuilleminii Namyslowski, Ann. Mycol. **8**: 152. *figs. 1–9.* 1910.

Zygorhynchus vuilleminii race *agamus* Namyslowski, Bull. Intern. Acad. Sci. Cracovie Cl. Sci. Math. Nat. Sér. B. **1910**: 479. *pl. 19.* 1911.

Zygorhynchus dangeardi Moreau, Bull. Soc. Bot. France **59**: LXVII, 717. *figs. 1–3.* 1912.

Zygorhynchus bernardi Moreau, Bull. Soc. Bot. France **60**: 256. 1913.

Zygorhynchus circinelloides Pispek, Acta Bot. Inst. Univ. Zagrebensis **4**: 94. *fig. 9.* 1929.

Zygorhynchus viridis Pispek, Acta Bot. Inst. Univ. Zagrebensis **4**: 96. *fig. 10.* 1929.

Zygorhynchus grisco-cinereus Pispek, Acta Bot. Inst. Univ. Zagrebensis **4**: 98. 1929.

Zygorhynchus vuillemini Namyslowski var. *dangeardi* Zycha, Kryptogamenfl. Mark Brandenb. **6a**: 95. 1935.

Zygorhynchus vuillemini Namyslowski var. *albus* Christenberry, Jour. Elisha Mitchell Sci. Soc. **56**: 342. *figs. 208–212.* 1940.

Zygorhynchus verruculosus Tehon, Trans. Ill. Acad. Sci. **36**: 109. *figs. 1–15.* 1943.

Colonies rapidly covering the surfaces of Petri dishes in less than 11 days with a height up to 1 cm or more, at first white, then Pallid Neutral Gray, or Pale Gull Gray to Deep Olive-Gray at the margin, upon aging Smoke Gray to Olive-Gray; colony reverse Olive-Buff and upon aging near Pale Olive-Buff to Deep Olive-Buff, showing irregular or cerebriform zonations; odor sweet and yeasty, sometimes musty, to none; sporangiophores from substrate mycelium erect or sometimes collapsed, hyaline, smooth to slightly roughened, with few septations which are found above origin of branches, usually without septations below sporangia, with zygospores and sporangia on same stalk, irregularly or sympodially branched, up to 15 μ in diam., not phototrophic; sporangia borne erect or in a nodding fashion, globose to slightly flattened, many-spored, white then gray or olive-gray in reflected light, 12–40 μ in diam., sometimes up to 70 μ; sporangial wall hyaline, smooth or encrusted, breaking, in some strains wall remaining almost intact and attached; columellae typically applanate although sometimes nearly globose, with a collar, faintly gray or brown in color, smooth, 6–8.6 $\times$ 8–25 μ, but sometimes up to 36 $\times$ 45 μ; sporangiospores smooth-walled, hyaline, with one or more oil droplets, typically with 1 at either end of spore, generally oblong to oval, sometimes bluntly triangular or almost reniform but never spherical, 2–3.3 $\times$ 3–6.5 μ; chlamydospores present or absent, in aerial mycelium, solitary or in twos, intercalary, typically oval in shape although other shapes are encountered, smooth, heavy-walled with homogeneous contents, granular or with oil droplets, up to 13 μ in diam., but occasionally up to 12 $\times$ 22 μ; oidia occasionally seen in substrate mycelium, terminal or intercalary, oval to nearly globose, thin-walled, contents granular and with oil droplets inside, to 13 μ in diam., but some up to 17 $\times$ 32 μ; zygospores 15–56 μ in diam., mostly 30–35 μ in diam., borne between unequal suspensors, typically just above substrate, but also throughout aerial mycelium, globose or slightly compressed, brown to dark brown, sometimes surrounded with droplets of liquid, roughened with projections (whose ends are blunt and sometimes recurved, appearing stellate at their base, and up to 5 μ in length, but typically less than 4 μ); smaller suspensors straight, 4–8 μ in diam.; larger suspensors recurved, gradually enlarged and somewhat constricted at attachment to zygospore, smooth to slightly roughened, empty or

sometimes contents with yellow droplets, wall hyaline to faintly brown-colored, 10–27 μ in diam.; azygospores occasionally present, dark brown, roughened, heavy-walled, to 18 μ in diam.; homothallic.

TYPE strain: NRRL 2660.

This is one of the most common species of the family Mucoraceae found in soils. In our experience *Z. moelleri* is isolated only from soil or soil-contaminated material. Studies have been made on isolates of this species secured from Illinois, South Carolina, Georgia, Wisconsin, New York, California, Indiana, New Hampshire, Florida, Michigan, Massachusetts, Brazil, England, Holland, New Zealand and Africa. The species appears to be cosmopolitan in distribution and is by far the most often encountered species in the genus *Zygorhynchus*.

The above description is based upon studies of various isolates grown for 5 to 12 days at 25° on SMA. Colonies of this species on Czapek's-solution agar are always very thin, showing only a few sporangiophores bearing sporangia, zygospores, or both. *Z. moelleri* on PDA shows typically low colonies which rapidly cover the surface of the agar. The colonies are dark gray, often broadly zonate and with the reverse colored gray. A few isolates, such as NRRL 1498, show more aerial growth than typical fresh soil isolates, and some strains, such as NRRL 1499, show lighter sporulation. On malt agar the surface of the agar is covered with mycelium which sometimes grows completely to the lid of the Petri dish. The number of zygospores is usually large.

In some cultures, such as NRRL 2659, the colonies on SMA are both gray and more or less papillate at the center. Outside this gray zone there is a raised zone rather white in color and at the margin the colony is again depressed and gray.

In strain A-7325 on Czapek's-solution agar, spines which reach a length of 6 μ are seen on the zygospores. Also in this strain the suspensors are rough on PDA, with zygospores very dark to almost black, but the larger suspensors taper gradually upward and not abruptly as in *Z. heterogamus*.

Strain NRRL 1493 is atypical because some of the sporangiospores on SMA, hay agar and malt agar are very short oval to almost globose, although many are oval in shape. However, only in this characteristic does it seem to be distinct from other strains and, therefore, is considered as a member of the species.

Some strains of *Z. moelleri* which have been under continued cultivation become much paler in color because of reduction of zygospore formation—for example, strains NRRL 1494 and NRRL 1496. Both were received in 1940 from the Blakeslee Collection, which, in turn, had

received NRRL 1496 before 1913 and NRRL 1494 in 1928 from Baarn. In addition, a culture received from Dr. W. L. Gauger of the University of Nebraska designated as "Z-2" shows this condition. The history of this isolate is unknown. Still other strains, NRRL 1500 and NRRL 1495, do not show any zygospores on SMA or PDA at 12 days. These strains were likewise from the Blakeslee Collection. NRRL 1495 came from R. Thaxter originally, the date not known, and NRRL 1500 from the Blakeslee Collection in 1940 without the source given. However, in all of the above strains the asexual means of reproduction are essentially like those of typical strains of *Z. moelleri*. Blakeslee stated that the strain now designated NRRL 1500 belonged in the *Z. moelleri* type but the exospore was different from that in *Z. moelleri*. Since zygospores are no longer produced, it has not been possible to check this characteristic.

Namyslowski (**1910**) described an organism which was similar to *Z. moelleri* except that it failed to form zygospores. He called it a race of *Z. vuilleminii* and named it *agamus*. This culture is maintained in our collection as NRRL 1497, which originally came from Namyslowski through the Baarn Collection. Study of this culture shows a strain similar to *Z. moelleri* but devoid of zygospores.

One isolate which we received from Mr. F. L. Raymond is stated by him to have been a sector from his isolate of NRRL A-5490. This culture, NRRL A-5491, shows spores and columellae as in *Z. moelleri* but no zygospores. It appears that strains can be selected which would fall in the genus *Mucor* were it not known that they are mutants of *Zygorhynchus*.

Zygorhynchus vuilleminii var. *albus* Christenberry was characterized by its white colony and the shorter spines of the zygospores. Moreau (**1912**) distinguished his new species *Z. dangeardi* from *Z. vuilleminii* and *Z. moelleri* primarily on the presence of four fused nuclei in the zygospore as compared to a much larger number of nuclei in the zygospores of "*Z. vuilleminii*" and *Z. moelleri*. Later Zycha (**1935**) reduced the species *Z. dangeardi* to varietal rank. We do not think it proper to attach any primary taxonomic importance to such fragmentary cytological details as the number of fused nuclei in the zygospore. Further, unless a cytological study of a number of strains of the species in question is made it is difficult to establish definitely the constancy of the number of nuclei in the different species. The type strain, which is carried as NRRL 2661, was received from Baarn as *Z. vuilleminii* var. *dangeardii* (Mor.) Zycha and although it possesses columellae, sporangia and sporangiospores like those of *Z. moelleri*, this strain has almost lost

its ability to form zygospores. Zygospores were found in this strain on malt agar but not on SMA, PDA, hay agar or corn-steep agar.

Namyslowski (**1910**) distinguished his new species *Z. vuilleminii* from *Z. moelleri* mainly on the basis of differences in size of sporangiospores and the projections of the zygospores. In the former species the average spore size is described as $4 \times 2\ \mu$ and in the latter $6 \times 3\ \mu$. The authors examined four different strains of *Z. moelleri*, two of them from the Blakeslee Collection, and many different strains of *Z. vuilleminii*, including the one from Baarn. We have found the size of sporangiospores in all these strains to vary from 4–6 μ in length and 2–4 μ in width. Larger spores rarely have been seen. Further, the size of zygospore projections in the two species is said to be 2–3 μ in *Z. vuilleminii* and 4–6 μ in *Z. moelleri*. In our observations, made on several different media, we have failed to see any difference in size of the projections in the two species. Their size ranges from 2–4 μ and rarely up to 5 μ. In none of the strains of *Z. moelleri* have we encountered zygosporic projections ranging exclusively from 4–6 μ. In addition to the difference in size of projections, a difference in arrangement of projections in the two species was also emphasized by Namyslowski (**1910**). We have experienced practically no difference in this characteristic either, for in both species the projections are borne singly as well as in groups. Taking the above facts into consideration, we believe it necessary to combine the two species, *Z. moelleri* Vuillemin and *Z. vuilleminii* Namyslowski, into the one species *Z. moelleri* Vuillemin, which has priority. Earlier Jensen (**1912**) and Zycha (**1935**) also considered the differences between the two species as very meager.

Lendner (**1908**) did not recognize the genus *Zygorhynchus* and therefore transferred the species *Z. moelleri* into *Mucor*, as *M. moelleri* (Vuill.) Lendner.

The species *Z. bernardi* Moreau appears to be a synonym of *Z. vuilleminii*, according to Zycha. Moreau's species was reported to have sporangiospores $2 \times 3\ \mu$ in size with zygospores 32–50 μ in diam. This species is considered to be a synonym of *Z. moelleri*.

Pispek described three species of *Zygorhynchus*, *Z. viridis*, *Z. circinelloides* and *Z. griseo-cinereus*, which were considered as synonyms of *Z. moelleri* and *Z. vuilleminii* by Zycha. *Z. circinelloides* was illustrated with applanate columellae, oval sporangiospores and typically *Z. moelleri*-formed zygospores. There is nothing stated in the description to indicate a difference except the nodding sporangia. This condition has been observed in our cultures and cannot be used as a species characteristic. The *Z. viridis* description does not indicate sporangiospore

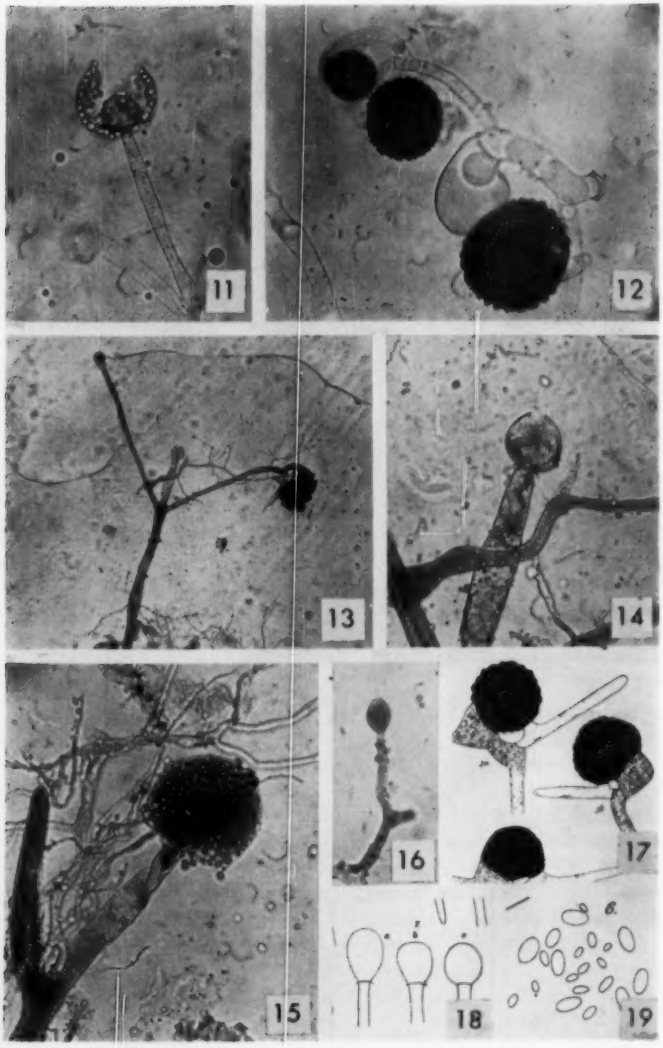
size, but the illustrations again show spores to be small and clearly oval in shape. There is nothing in the measurements or illustrations to lead one to believe this species is anything but another isolate of *Z. moelleri*. The last species, *Z. griseo-cinereus*, was not illustrated and was but poorly described. The sporangiospores are ellipsoid, 3.5 × 2–2.5 μ, and as suggested by Zycha the name is still another synonym of *Z. moelleri*.

The last species reduced to synonymy is *Z. verruculosus* Tehon. This species was isolated from the roots of a diseased elm in Peoria. Examination of a prepared herbarium slide showed smooth, hyaline sporangiophores measuring about 8.5 μ in diam. and globose sporangia about 40 μ in diam., with walls breaking and with applanate columellae. The smooth spores were oval in shape but of the size found in *Z. moelleri*. The zygospores had projections up to 4.5 μ in length. Measurements reported by Tehon are similar to those encountered in isolates of *Z. moelleri*. Attempts to isolate this species in the Peoria area gave only additional strains of *Z. moelleri*. It is concluded that this was also a strain of *Z. moelleri*.

4. **Zygorhynchus californiensis** sp. nov. FIGS. 8–10

Coloniae plus quam 1 cm altae, pallide murinae usque cinereae, reverso flavido; odor dulcis, valde eo Actinomycetum similis; sporangiophora irregulariter vel sympodialiter ramosa, zygosporas sporangiaque ferentia; sporangia atro-grisea, globosa, rumpentia, 15–60 μ; columellae dorsiventraliter applanatae, collariatae, 6.5–26 × 8–35 μ; sporangiosporae glabrae, globosae, hyalinae, 2.2–4.5 μ; zygosporae 20–47 μ, globosae, brunneae vel atro-brunneae, spinis usque ad 4.5 μ longis praeditae. E solo isolatus, California.[3]

Colonies often over 1 cm in height, later collapsing at about 1 month, growth rapid, covering surface of Petri dish in less than 10 days, Pale Mouse Gray to Gull Gray, at 1 month Grayish Olive to Drab; reverse side of colony with definite yellow shades near Dark Olive-Buff, and at 1 month Deep Olive-Buff; odor sweet and strongly actinomycete-like; sporangiophores arising from substrate, with septa above origin of branches, hyaline, smooth-walled but with branches bearing secondary sporangia or zygospores typically roughened, erect, branching sympodially below as seen on Czapek's-solution agar, but often irregularly, up to 13 μ in diam.; true stolons and rhizoids absent; sporangia at first often nodding, later borne erect, at first white then blackish-gray in reflected light, brown in transmitted light, without septa below sporangia, formed earlier than the zygospores, remaining intact in mounts, hence breaking only with difficulty, globose to slightly elongate, in some cases almost *Absidia*-like, in others abortive or empty, 15–60 μ in diam.; sporangial wall seldom encrusted, transparent; columellae always applanate, smooth-walled, light tan or very light brown in color, with most of sporangial



FIGS. 11–12. *Zygorhynchus heterogamus.* FIG. 11. Sporangiophore, showing broken sporangium, columella, and sporangiospores. FIG. 12. Zygospores. FIGS. 13–16. *Z. heterogamus,* from Vuillemin's type slide. FIG. 13. Sporangiophore,

wall always remaining attached, often with sporangiospores adhering, 6.5–26 × 8–35 μ in size; sporangiospores smooth, globose or slightly irregularly globose, hyaline or very faintly yellow, typically with one vacuole or oil droplet in center, 2.2–4.5 μ in diam.; chlamydospores not seen except in old cultures and then oval and intercalary; substrate mycelium empty except for a few colorless oil droplets; zygospores found throughout the aerial part of colony, regularly globose, 20–47 μ in diam., but typically 30–38 μ, brown to dark brown but never black, roughened with blunt projections up to 4.5 μ in length, but usually only to 3.5 μ; larger suspensors smooth or roughened, hyaline, gradually enlarging to a diam. of 12–24 μ, then slightly constricted at attachment to zygospore; smaller suspensor very short, straight, about 5 μ in diam.; homothallic.

The above description is based on studies of the TYPE strain, NRRL 2658, grown on SMA for periods of 10–14 days. This culture was isolated from soil collected at Yosemite, California. Only the single type strain has been examined.

This species, like other *Zygorhynchus* species, grows as a thin transparent colony on Czapek's-solution agar. Its sporangiophores branch sympodially, cymosely and irregularly. Of especial interest are the extremely long sporangiophores, which extend for considerable distances just above the surface of the agar to give the effect of stolons. Upon PDA, the same type of sporangiophores is seen. Abundant production of zygospores and sporangiospores occurs on most media.

A peculiar characteristic of this species is the strong, actinomycete-like odor formed along with the sweet "yeasty" odor characteristic of many *Mucors*. This odor is pronounced upon SMA, malt agar, and Czapek's-solution agar. After 1 month, the cultures have lost this peculiar odor although they still retain a foul odor.

Zygorhynchus californiensis is related to those species in the genus which possess globose spores. However, it differs from *Z. exponens* by having smooth spores and sporangia which never show dehiscence in place. It is also distinct from *Z. heterogamus* because of the regular globose sporangiospores, the brown instead of black zygospores, the larger suspensor which always tapers gradually to its maximum size, the typically larger sporangia and the sporangiophores which also are much longer as seen on Czapek's-solution agar.

We have named it *Z. californiensis* because of its type locality.

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zygospore, sporangium, and columella. FIG. 14. Higher magnification of the columella shown in fig. 13. FIG. 15. Sporangium and sporangiospores. FIG. 16. Chlamydospore. FIGS. 17–19. *Z. japonicus*, after Kominami. FIG. 17. Zygospores. FIG. 18. Columellae. FIG. 19. Sporangiospores.

5. ZYGORHYNCHUS HETEROGAMUS (Vuill.) Vuillemin, Bull. Soc. Mycol. France **19**: 117. 1903. FIGS. 11–16

Mucor heterogamus Vuillemin, Bull. Soc. Bot. France **33**: 238. 1886; Bull. Soc. Sci. Nancy II. **8**: 50. *pl. II, figs. 27–48.* 1887.

Mucor neglectus Vuillemin, Bull. Soc. Sci. Nancy II. **8**: 83. *pl. IV, figs. 66–70.* 1887.

Zygorhynchus phosphoreus Pispek, Acta Bot. Inst. Univ. Zagrebensis **4**: 97. 1929.

Colonies often up to 1 cm in height, sometimes very low with irregular margins, in some strains covering the surface of the media in Petri dishes in less than 10 days on SMA, white at first, then Smoke Gray, Dark Neutral Gray or Deep Grayish Olive with center of colonies sometimes depressed or overgrown with white mycelium; colony reverse Dark Olive-Buff, Grayish Olive or Deep Olive-Buff, hence with a yellow tint; odor faint to sweetly fragrant; sporangiophores arising from substrate, hyaline, smooth to slightly roughened, typically erect although sometimes collapsed, with septations above origin of branches and occasionally elsewhere, sympodially or irregularly branched, often with sterile branches, with both sporangia and zygospores, often producing a small number of sporangia, not phototrophic, up to 18 μ in diam.; sporangia hyaline to faintly yellow in reflected light, borne erect or nodding, globose, breaking or sometimes deliquescing, many-spored, 15–55 μ, typically not more than 35 μ in diam.; sporangial wall transparent, sometimes encrusted; columellae applanate to almost globose, hyaline to faintly gray- or brown-tinted, with collar to which much of the sporangial wall is sometimes attached, 4.5–22 $\times$ 6.5–26 μ; sporangiospores irregularly short oval (a few globose), smooth, thin-walled, hyaline, with one or many vacuoles, usually not with two distinct vacuoles, 2–4 $\times$ 4–6.5 μ, occasionally up to 5 $\times$ 8 μ; chlamydospores in substrate mycelium and occasionally in aerial mycelium, from few to very numerous, intercalary or more rarely terminal, typically single, hyaline, thick-walled, globose to oval or even elliptical, and often with rather bifurcate ends, with contents vacuolate and granular, 6.5–21 μ in diam.; zygospores 30–70 μ in diam., formed throughout the colony but most abundant just above substrate, globose to slightly compressed between the suspensors, dark brown to black with the larger mature zygospores almost always black, covered with blunt projections up to 4.5 μ in length; suspensors unequal; smaller suspensors hyaline, smooth, straight, not inflated, 6–13 μ in diam.; larger suspensors hyaline to light gray or brown, with granular contents, smooth to faintly roughened, bent, enlarging upward and abruptly inflated into a globose or short oval shape and then constricted at attachment to zygospore, 28–57 μ in diam.; homothallic.

TYPE: Vuillemin's slide labeled "*Mucor heterogamus* P.V. Zygospore Mis de Pain 17–3–86."

The above description is a composite one based upon a study of five different strains grown on SMA for 10–11 days at 25°.

When these strains are grown on Czapek's-solution agar, growth is scant but sporangiophores bearing both sporangia and zygospores can be very clearly seen. The sporangiophores growing on this medium are consistently roughened. On PDA and malt agar growth is luxuriant and abundant zygospore formation occurs.

The strains of this species studied were NRRL 1489, NRRL 1490 and NRRL 1491 from the Blakeslee Collection, which have been maintained in culture at least since 1924; strain NRRL 1616, which came to our collection in 1940 as Harvard University Collection strain No. 202; and one freshly isolated strain obtained from William Bridge Cooke as *No. 1957* from Lytle Creek, Clinton County, Ohio, collected from soil subjected to flooding.

The variations in colony appearance of the isolates of *Z. heterogamus* are quite marked when grown upon SMA. The reverse side of the colony ranges in color from a grayish-yellow, such as seen in NRRL 1491, to strains with practically no yellow color. The colony surfaces are very variable. In NRRL 1489 the colonies are greatly raised and white with the colony margin irregular. When the colony is a month old, it has apparently collapsed and assumed a dark gray color. In such strains as NRRL 1490, the colonies are gray, low, irregular of margin and at 1 month have failed to cover the surface of the agar in a Petri dish and have assumed a very dark gray color. Other strains, such as NRRL 1616, have uniformly gray colonies which are low but unlike NRRL 1490 soon cover the entire surface of a Petri dish. Another strain which was recently isolated was similar to NRRL 1616, but showed a large amount of overgrowth of white mycelium in the center of the colony.

On PDA, NRRL 1489 tends to show moist areas in the center of the plate and a gray color both above and on the reverse side of the colony. The remaining strains studied are quite uniform on PDA, producing low, gray colonies and covering all the surface of the agar. In no case are moist areas present.

The type material used by Vuillemin was studied directly from four slides that he had prepared in 1886. We are most grateful to Professors Mangenot and Helluy of the École National Supérieure Agronomique, Nancy, France, through whose efforts we were able to study

the original slides. The four had been prepared by Vuillemin during his study of the development of the zygospores; one shows the mature condition. These slides show some of the original material from which Vuillemin illustrated his paper published in 1887. An examination of the slides reveals both zygospores and sporangia on sporangiophores at least 1,400 μ in length with a width of up to 20 μ. The sporangiophores have smooth or minutely roughened walls and are generally sympodially branched with septations above the origin of the branches. Sporangia are globose, 55 μ in diameter, hyaline and smooth, containing many sporangiospores and appearing to have been borne erect; columellae are globose with collars up to 27 μ in diameter. Sporangiospores are short-oval to globose with smooth walls and uniform contents. They measure 3–5.5 μ in length and 2.1–4 μ in width. Chlamydospores are present, both terminal and intercalary, in the substrate mycelium and are borne singly. They are smooth, heavy-walled, oval in shape and 13 × 17 μ. Thus, this material is almost identical with that found in our living cultures. The zygospores in Vuillemin's material are borne between unequal suspensors with the larger suspensors up to 46 μ in width and with the same form as indicated in our description. The smaller suspensors are straight and about 10.7 μ in diameter. Mature zygospores are present but all have been crushed so that it is difficult to determine accurately their size, although a crushed one measures 120 μ. Their shape is globose and the color dark brown in the stained preparations. Projections are about 4.5 μ in length, but in some mature crushed zygospores lengths of 12 μ are encountered. This does not agree with our measurements. The material was undoubtedly grown in impure culture and then fixed and stained. These factors would account for the differences. Because we have encountered so much variability in the length of the projections on the zygospores, we consider this material and ours to belong to the same species.

Vuillemin in 1886 first described the species as *Mucor heterogamus*, but in 1903 made the combination *Z. heterogamus* (Vuill.) Vuillemin. In 1887 Vuillemin also described *M. neglectus*, which he later (**1907**) stated was only a form of *Z. heterogamus* which produced azygospores.

Zygorhynchus phosphoreus Pispek was described briefly and without illustrations. Zycha considered it to be a synonym of *Z. heterogamus*. We concur with this conclusion since the measurements agree with those of their strains and especially because the spores were both globose and elliptical in shape, a condition not encountered in the only other possible species to which it might belong, *Z. moelleri*. Pispek apparently did not report *Z. heterogamus* but he did report *Z. moelleri*.

6. ZYGORHYNCHUS MACROCARPUS Ling-Young, Rev. Gén. Bot. **42**: 150. 1930. *fig. 1.*

The following description is a summary of the data given by Ling-Young in the original description of *Z. macrocarpus:*

Colonies at first yellow, then brownish gray, mostly 1–2 mm high (on bread up to 3–4 mm); sporangiophores sympodially or unequally branched, branches erect, bent or circinate, 15–20 μ in width; sporangia globose, a little depressed, at first pallid, then blackish gray, 50–100 μ in diam., smaller ones down to 25 μ in diam.; sporangial wall gray, roughened, breaking in water to leave a basal collar; columellae smooth, free or slightly adnate, applanate, occasionally globose, 20–40 μ high, 25–45 μ wide (in small sporangia all the way down to 8 μ in diam.); sporangiospores ovoid, smooth, slightly yellow, often with 1 or 2 droplets, 2.5–3 $\times$ 4.5–5, rarely 7 μ in length; chlamydospores abundant, spherical, variable in size, 5–25 μ in diam., present in submerged mycelium; zygospores globose, 50–100 μ in diam., at first minutely tuberculate, pale brown, then with black projections; projections acute, often recurved, stellate at the base, 6–7 μ in length, 8–10 μ in width; suspensors unequal, the smaller straight, the larger recurved, swollen, pyriform, short; azygospores rare.

Our observations are as follows:

Colonies up to 4 mm in height, covering the agar in Petri dishes in less than 11 days on SMA at 25°, Light Olive-Gray and at 1 month Grayish Olive; colony reverse Pale Olive-Buff and remaining this color at 1 month; odor none; sporangiophores from substrate, hyaline, occasionally smooth-walled but typically roughened, with septa sometimes below sporangia and at origins of branches, erect, branching mostly irregularly although sometimes sympodially, with branches often seen arising at right angles to each other on Czapek's solution agar, up to 17 μ in diam.; not phototrophic; rhizoids and stolons absent; sporangia colorless to faint yellow-brown in reflected light, borne in a nodding fashion when young but erect when mature, deliquescing, globose, many-spored, up to 75 μ; sporangial wall smooth, hyaline; columellae smooth, hyaline, mostly globose or short oval, rarely applanate, with collar only at region of attachment, 15–42 μ in diam.; sporangiospores globose to short oval, smooth, hyaline, usually with 1–2 vacuoles, 2–4.5 μ in diam. where globose, up to 3.3 $\times$ 4.5 μ where oval; chlamydospores abundant in substrate, terminal or intercalary, globose to oval with ends often apiculate, smooth, heavy-walled, up to 30 $\times$ 42 μ; zygospores absent, except as noted by Ling-Young.

The only culture examined of this species was NRRL 2663, which was received from the Centraalbureau voor Schimmelcultures, Baarn,

Holland, which had received it from Ling-Young; and therefore it may be the type strain. However, it had lost its ability to form zygospores on any medium on which the strain was grown. On Czapek's-solution agar the colonies are thin and colorless although sporangia are formed in abundance. On PDA the light gray colonies reach nearly to the lids of the Petri dishes; the surface of the agar is covered, and the colony reverse is near cream in color. No odor was produced in this medium.

Ling-Young isolated this species twice from peaty soil in France on the slope of Sud du Puy Ferrand at 1,600 meters. It was said to be related to *Z. dangeardi*. The zygospores shown by Ling-Young are quite like those seen in *Z. heterogamus*, including their black color when mature.

The culture we examined, besides having no zygospores, failed to show the applanate columellae. The spore shape was globose, as well as oval, while Ling-Young illustrates regularly oval or even elliptical sporangiospores. In addition, we encountered no sporangia as large as 100 μ as did Ling-Young. It must be concluded that NRRL 2663 is not this species, or if it is, it has changed radically and can no longer be considered typical. Attempts to locate type material prepared in 1930 were unsuccessful. However, *Z. macrocarpus* is sufficiently illustrated and described that it should be identifiable if again encountered. We are, therefore, recognizing it as a species until evidence is presented to the contrary.

7. ZYGORHYNCHUS JAPONICUS Kominami, Mycol. Centbl. **5**: 3. 1914. *figs. 1–7.* FIGS. 17–19

Sporangiophores uncolored, smooth, 9–15 μ in width, variable in length, rarely more than 1 cm in height; sporangia spherical, yellowish, deliquescing, up to 56 μ in diam.; columellae pyriform, rarely spherical, free, smooth, uncolored, 30–45 × 20–30 μ wide; sporangiospores elliptical, thin-walled, smooth, uncolored, very variable in size, 3–10 × 1.5–6 μ; chlamydospores rather rare, oval, smooth, 25 × 40 μ; zygospores formed on sporangiophores or on mycelial filaments branched sympodially, heterogametic, yellow then brown to black, roughened by projections, 68 μ in diam. with a maximum of 80 μ; projections 2–3 μ in length, arranged in groups separated by clear areas; smaller suspensor straight and short; larger suspensor long, curved, rarely ending in a pyriform swelling and usually with a more or less long protuberance; homothallic.

TYPE: Unknown.

Isolated from soil from Kamakoura, Japan.

The above description is taken from Kominami's paper. *Z. japonicus* is distinct from the other species in the following respects: by the range

in size of the sporangiospores, which is much greater than that encountered in any other species with elliptical spores; secondly by the peculiar formation of the zygospores, in which the larger suspensor has a more or less elongate projection in the region of its curvature; and thirdly, by having pyriform columellae which are rarely globose and never applanate. It, therefore, is most closely related to *Z. moelleri* in having oval to elliptical sporangiospores but can be distinguished by any one of these other characteristics.

DOUBTFUL SPECIES

ZYGORHYNCHUS HENNEBERGII Naumov, Clés des Mucorinées, p. 58. 1939, nom. nud.

The only available description concerning this species is given in Naumov's key (**1939**). This appears to be very incomplete and furthermore indicates no earlier publication, but only the entry of the name "in schedulis." It is stated that *Z. hennebergii* had zygospores 60–120 μ in diam., with a colony height to 3–5 mm and with sporangia 40–60 μ in diam. The sporangiospores are stated to be 6–7 × 3–3.5 μ in size. This would indicate a species near *Z. macrocarpus*. Dr. N. A. Komarnitzky at Moscow State University informed up that neither a culture nor a herbarium specimen can be located in Russia.

SUMMARY

The genus *Zygorhynchus* Vuillemin, which has had 17 species and varieties described in it, has been studied. It is concluded that there are six species and one variety, including one new species, *Z. californiensis*, and a new variety, *Z. exponens* var. *smithii*. In addition *Z. exponens* Burgeff, *Z. moelleri* Vuill., *Z. macrocarpus* Ling-Young, *Z. heterogamus* (Vuill.) Vuill. and *Z. japonicus* Kominami are recognized. *Z. vuilleminii* Namysl. has been reduced to synonymy under *Z. moelleri*, and *Z. hennebergii* Naumov is considered as a doubtful species.

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HOMOTHALLISM IN AN UNDESCRIBED SPECIES OF COCHLIOBOLUS AND IN COCHLIOBOLUS KUSANOI [1]

E. S. Luttrell and Clark T. Rogerson [2]

(with 4 figures)

Perithecia of seven species of *Cochliobolus*, representing perfect stages of *Helminthosporium* spp., have been produced in culture by isolates from diseased tissue or from single conidia. The fact that single-ascospore analysis subsequently demonstrated heterothallism in two of these species, *C. heterostrophus* (Drechs.) Drechs. and *C. sativus* (Ito & Kurib.) Drechs. ex Dastur, as well as in *C. nodulosus* Luttrell, raised some doubt as to the occurrence of homothallism in this genus (Luttrell, **1957, 1958**). Cultural studies of single-ascospore isolates, however, have now confirmed homothallism in one of these species, *C. kusanoi* (Nisik.) Drechs. ex Dastur, and also in an apparently undescribed species isolated from air-borne spores in Kansas. The new species is described as follows:

Cochliobolus homomorphus sp. nov.

Peritheciis superficialibus vel innatis, nigris, subglobosis, 469–649 μ altis, 524–731 μ latis, rostro cylindrico, 152–221 × 69–83 μ praeditis; ascis unitunicatis, cylindraceis, breve stipitatis, apice rotundatis, octosporis, pseudoparaphysatis, 126–224 × 13–20 μ; ascosporis hyalinis, filiformibus, utrinque acutatis, 134–304 ×318–4.6 μ, 6–9-septatis, ad septa non vel leniter constrictis, spiraliter convolutis.

Helminthosporium homomorphus sp. nov. status conidicus

Conidiophoris praelongis, fluexuosis, cylindraceis, simplicibus vel parce ramosis, brunneo-fuligineis, sursum pallidioribus, 56–622 × 5.6–7.8 μ, 4–47-septatis; conidiis acropleurogenis, brunneo-fuligineis vel brunneis, anguste ellipsoideis, oblongis, vel oblongo-clavatis, rectis, utrinque obtusis, non constrictis, 25–42 × 10–13 μ, 3–6-septatis, tenue tunicatis, hyphis polaribus germinantibus.

Hab. ex aero, cultura in stramine *Hordei vulgaris*.

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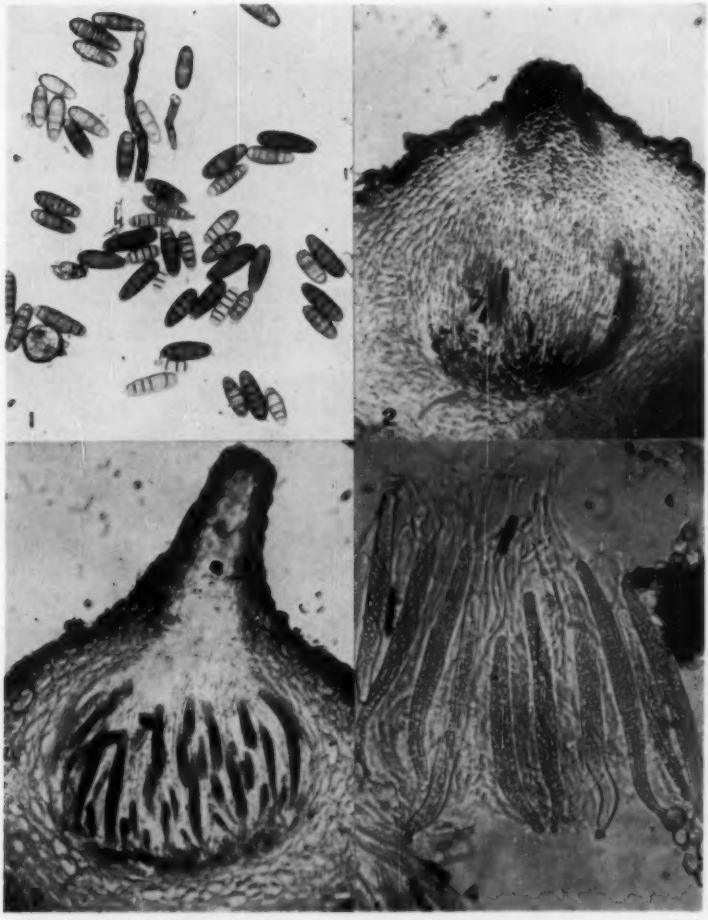
Specimens (*Luttrell No. 6002,* TYPE) have been deposited in the National Fungus Collections, Beltsville, Maryland; the Herbarium of the Royal Botanic Garden, Kew; and the Herbarium of The New York Botanical Garden. Cultures have been placed in the American Type Culture Collection and in the Centraalbureau voor Schimmelcultures.

COCHLIOBOLUS HOMOMORPHUS

The original culture (*C. T. Rogerson No. B227-14*) of *Cochliobolus homomorphus* was obtained from air with a Pady-Rittis sampler located 175 feet above ground at Kansas State College on June 11, 1957. This culture was transferred to plates of water agar sprinkled with ground, propylene-oxide-sterilized wheat straw. In these plates it produced both *Helminthosporium* conidia and *Cochliobolus* perithecia.

Production of Perithecia.—From the original culture 30 single-conidium (No. 1268-1 to 30) and 34 single-ascospore (No. 1266-1 to 34) isolations were made. On 20 per cent V-8-juice agar all isolates produced uniform colonies of dark-gray, cottony mycelium with linear radial growth rates at 25° C over a six day period of 4.0 to 4.2 mm per day. When the single-ascospore isolates were mated in various combinations, all pairings produced perithecia. In subsequent tests perithecia were produced abundantly by every single-conidium and single-ascospore isolate individually.

Perithecia developed readily on various plant parts sterilized with propylene oxide and partially immersed in or placed on the surface of Sachs's agar plates. These included segments of barley or wheat culm, wheat spikelets, and alfalfa, rescue grass, and rye grass leaves. Perithecia developed on the plant parts and in the surrounding agar. Perithecia on corn grains surface sterilized with Clorox remained sterile, although mature asci were present in perithecia in the surrounding agar. Perithecia developed also in plates of 5, 10, and 20 per cent V-8-juice agar and in potato-dextrose, oat-meal, and barley-straw-decoction agar. Five per cent V-8-juice agar was a favorable medium since perithecia formed abundantly and there was less aerial mycelium. Many of the perithecia were completely immersed in the agar. Perithecia matured within three weeks in plates kept in the laboratory and exposed to diffuse daylight. Few or no perithecia were present in plates in incubators at 20° and 25° where there was little exposure to light. When wheat heads and straw previously sterilized with propylene oxide were inoculated and placed out of doors in wire baskets in April, a few perithecia developed on the heads within two months, but none formed on the straw.



FIGS. 1–4. *Cochliobolus homomorphus.* FIG. 1. Conidia and conidiophore. FIG. 2. Section of an immature perithecium showing young, uninucleate asci in the pseudoparaphysate centrum and an early stage in formation of the beak. FIG. 3. Section of mature perithecium. FIG. 4. Mature asci and pseudoparaphyses.

When agar plates were inoculated at a single point in the center, perithecia developed chiefly along the margin of the colony as it approached the edge of the plate. Sterile glass rods partially embedded in the agar and the use of one or two layers of sterile glass slides placed in the bottom of the Petri dish to vary the depth of the agar medium had no effect on perithecium production. Perithecia developed earlier throughout the plates when they were inoculated at numerous points. However, perithecia were not produced along the margins of colonies when growth was inhibited by colonies of *Penicillium* sp. or bacteria inoculated on one side of the plate.

Morphology.—Cochliobolus homomorphus produced conidia sparsely on all media. Barley or wheat culm and barley-straw-decoction agar were the most favorable media. The conidiophores were very long, contorted, simple or sometimes with short branches, cylindrical, pale to moderately dark fuliginous brown, geniculate, and provided with circular conidial scars. They measured 56–622 × 5.6–7.8 μ and were 4–47-septate. Conidiophores formed on inoculated wheat straw placed out of doors measured 62–81 × 5.6–7.0 μ and were 4–20-septate.

Conidia formed singly at the tip of the conidiophore but became lateral as a result of a subterminal outgrowth of the conidiophore, which formed a new growing point and thus produced a succession of terminal conidia. The conidia (FIG. 1) were broad ellipsoid, oblong, or clavate-ellipsoid, widest in the upper third, straight, pale to moderately dark brown, or fuliginous brown, 25–42 × 10–13 μ, 3–6-septate, and thin-walled with a small circular hilum included within the contour of the wall at the base. They germinated by polar germ tubes. The germ tubes often developed directly into conidiophores producing secondary conidia.

Perithecia (FIG. 3) were superficial or immersed, black, globoid, 469–649 μ in height, and 524–734 μ in diameter, with a broad cylindrical beak measuring 152–221 × 69–83 μ. The centrum (FIG. 2) was composed of persistent, filiform pseudoparaphyses among which the asci developed. The asci (FIG. 3, 4) were unitunicate, cylindrical with a short stipe, 126–224 × 13–20 μ, and 8-spored. The ascospores were hyaline, filiform, 134–304 × 3.8–4.6 μ, 6–9-septate, often slightly constricted at the septa, and coiled in a helix in the ascus. In water mounts the ascus split across the apex, and the ascospores emerged in a group, slowly untwisting and separating.

COCHLIOBOLUS KUSANOI

Material of *Cochliobolus kusanoi* was obtained from a single collection by C. T. Rogerson, *No. R3835,* on leaves of *Eragrostis pilosa* (L.)

Beauv., Kansas State College, August 16, 1956 (Rogerson, **1958**). From this collection, five single-conidium isolations were made. When these five isolates were inoculated singly onto fragments of wheat culm sterilized with propylene oxide and partially immersed in plates of Sachs's agar (Luttrell, **1958**), all produced mature perithecia after three weeks exposure to normal diffuse daylight and fluctuating temperatures in the laboratory. From the perithecia produced by one of these isolates (No. 1263-4), 37 single-ascospore isolations were made.

All single-ascospore isolates (No. 1272-1 to 37) were nearly identical in colony characteristics. Colonies consisted of a fluffy, gray mycelium with irregular dendritic margins. Conidia developed abundantly on the aerial mycelium. Linear radial growth rates on plates of 20 per cent V-8-juice agar at 25° over a period of six days varied from 2.9 to 3.5 mm per day. All isolates produced numerous perithecia when transferred singly to sterilized wheat culm. No perithecia developed in V-8-juice-agar plates. Fertile perithecia were produced on surface sterilized corn grains embedded in Sachs's-agar plates, but they were abnormally large, globular structures lacking beaks. The most favorable medium for normal development of perithecia was segments of wheat or barley straw sterilized with propylene oxide and partially immersed in plates of Sachs's agar.

On agar plates some isolates, from ascospores as well as conidia, produced sectors of white mycelium lacking conidia. Isolates from these white sectors failed to produce either perithecia or conidia on sterilized wheat straw. During periods of storage on agar slants there was a tendency toward replacement of the original strains by sterile white strains.

EFFECT OF LIGHT ON DEVELOPMENT OF PERITHECIA

Since perithecia of both *Cochliobolus homomorphus* and *C. kusanoi* developed poorly in incubators at 20° and 25° under conditions most favorable for the production of perithecia in *C. nodulosus* (Luttrell, **1957**) but formed abundantly in plates kept in the laboratory, experiments were designed to determine the effect of light and temperature on perithecium formation in these two homothallic species in comparison with two heterothallic species, *C. nodulosus* and *C. heterostrophus*.

Plates of five per cent V-8-juice agar, propylene-oxide-sterilized wheat straw in Sachs's agar, and corn grains surface sterilized with 10 per cent Clorox and embedded in Sachs's agar, were inoculated with the following isolates: *Cochliobolus homomorphus* No. 1266-33, *C. ku-*

sanoi No. 1263-4, *C. nodulosus* No. 1208-58 × No. 1208-86, and *C. heterostrophus* No. 1260 × No. 1261. Half of the inoculated plates were kept on benches in the laboratory exposed to normal diffuse daylight and fluctuating temperatures; half were placed in an incubator at a constant temperature of 25°, where they were exposed to fluorescent light for an 18 hour day. Half of the plates in each location were enclosed in metal cans to exclude all light. Observations were made at the end of four weeks. Perithecia of *C. homomorphus* and *C. kusanoi* developed only in plates exposed to light, both in the laboratory and in the incubator. *C. nodulosus* and *C. heterostrophus* produced mature perithecia under all conditions. Perithecia of all species developed more rapidly at a constant temperature of 25° than at fluctuating lower temperatures.

When cultures of *Cochliobolus homomorphus* on five per cent V-8-juice agar were exposed to light in the laboratory for five days until the first perithecial initials were visible and then placed in total darkness for three weeks, mature perithecia developed. A few perithecia matured also in cultures kept in total darkness, exposed to diffuse daylight for six hours on the fifth day, and returned to total darkness for three weeks.

Light had no apparent effect on the production of conidia by any of these species.

DISCUSSION

Nisikado's (**1928**) report of the production of perithecia by a single-conidium isolate of *Cochliobolus kusanoi* suggested homothallism in this species, although the possibility remained that his results might be explained by heterocaryosis (Luttrell, **1957**). However, the production of perithecia by each of 37 single-ascospore isolates seems adequate evidence that *C. kusanoi* is homothallic. *C. homomorphus*, like *C. kusanoi*, differs from the three species that have previously been subjected to single-ascospore analysis (Tinline, **1951**; Nelson, **1957**; Luttrell, **1957**) in that it is homothallic. It differs also from most species in that it forms perithecia readily on agar media, although Matsumoto (**1952**) found that *C. stenospilus* (Carpenter) T. Matsu. & Yamamoto likewise produced perithecia on several agar media.

Shoemaker (**1955**) reported that perithecia of the heterothallic species *Cochliobolus sativus* developed best in total darkness. Results with two additional heterothallic species, *C. heterostrophus* and *C. nodulosus*, are in agreement with those of Shoemaker to the extent that perithecia matured at least as well in total darkness as in light. On the other hand, light was essential for the formation of perithecia by both of the

homothallic species, *C. homomorphus* and *C. kusanoi*. In *C. homomorphus* light apparently affected the initiation rather than the maturation of perithecia, although perithecia developed best when exposed to light throughout the period of their development.

Cochliobolus homomorphus appears to be most closely related to *C. tritici* Dastur (Dastur, **1942**), which was isolated from "black point" wheat grains in India. It differs from *C. tritici* in its larger perithecia, slightly narrower asci and ascospores, and smaller, fewer-septate, clavate-ellipsoid rather than ellipsoid conidia. These differences are minor. However, the basis for the recognition of many other species of *Cochliobolus* is hardly more satisfactory. It is evident that this genus must be critically revised when additional data on both the perfect and imperfect stages become available. The following improvised key indicates the relationship of *C. homomorphus* to other species of *Cochliobolus*.

KEY TO SPECIES OF COCHLIOBOLUS

1. Maximum length of ascospores less than 250 μ.....2
1. Maximum length of ascospores more than 250 μ.....5
2. Maximum diameter of asci 14 μ.....*C. sporoboli*
2. Maximum diameter of asci 17–20 μ.....3
3. Ascospores 140–230 μ long, diam. of perithecia 200–250 μ.....*C. boutelouae*
3. Maximum length of ascospores 170–193 μ, diam. of perithecia more than 300 μ.....4
4. Conidia long obclavate, 3–7-septate.....*C. nodulosus*
4. Conidia short obclavate, 1–4-septate.....*C. kusanoi*
5. Maximum length of ascospores more than 400 μ.....*C. miyabeanus*
5. Maximum length of ascospores less than 375 μ.....6
6. Minimum diam. of asci more than 30 μ.....*C. sativus*
6. Minimum diam. of asci less than 30 μ.....7
7. Minimum diam. of asci less than 20 μ.....8
7. Minimum diam. of asci more than 20 μ.....9
8. Diam. of perithecia 172–406 μ, asci 80–228 × 13–34 μ, ascospores 125–302 × 3.8–7.6 μ, conidia 46–84 × 11–15 μ, 5–9-septate.....*C. tritici*
8. Diam. of perithecia 524–731 μ, asci 126–224 × 13–20 μ, ascospores 134–304 × 3.8–4.6 μ, conidia 25–42 × 10–13 μ, 3–6-septate.....*C. homomorphus*
9. Diam. of perithecia 400–600 μ.....*C. heterostrophus*
9. Diam. of perithecia 220–448 μ.....10
10. Diam. of perithecia 220–315 μ, ascospores 5–9-septate.....*C. setariae*
10. Diam. of perithecia 238–448 μ, ascospores 4–12-septate.....*C. stenospilus*

SUMMARY

A new species, *Cochliobolus homomorphus* Luttrell & Rogerson, with the imperfect stage *Helminthosporium homomorphus* Luttrell & Rogerson, differing from *C. tritici* Dastur in its larger perithecia, narrower asci

and ascospores, and smaller, fewer-septate, clavate-ellipsoid conidia, developed in a culture isolated from air in Kansas. Single-ascospore analysis demonstrated that both *C. homomorphus* and *C. kusanoi* (Nisik.) Drechs. ex Dastur are homothallic. Light was essential for the formation of perithecia in these two homothallic species; whereas perithecia of two heterothallic species, *C. nodulosus* Luttrell and *C. heterostrophus* (Drechs.) Drechs., developed as well in total darkness.

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A NEW SPECIES OF ABSIDIA FROM CALIFORNIA

G. F. Orr and O. A. Plunkett

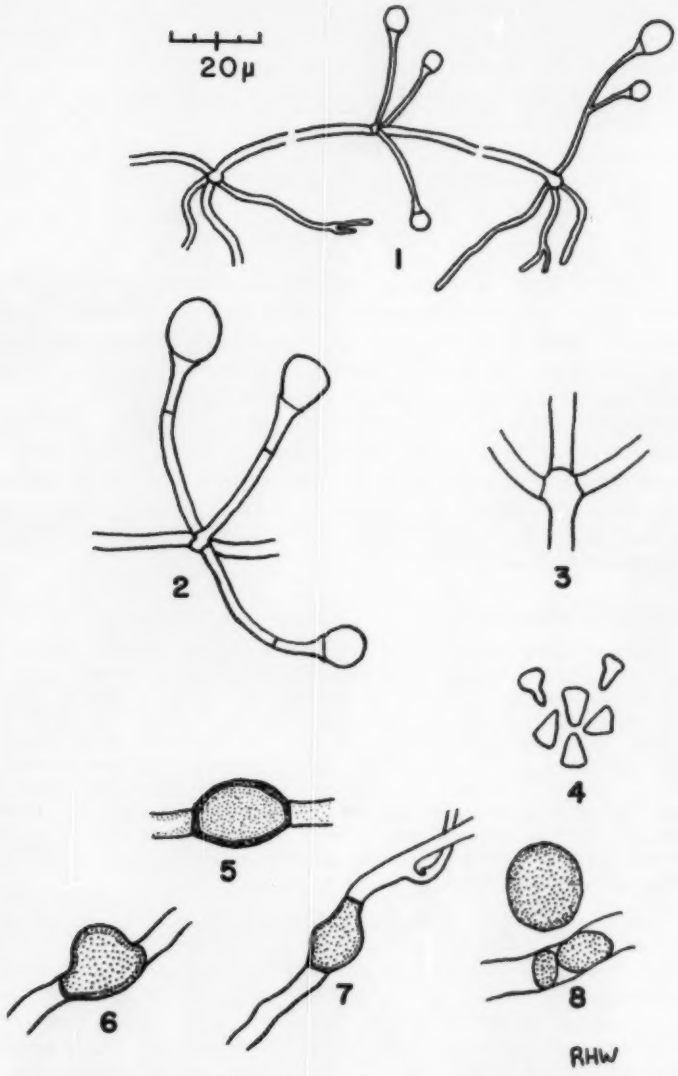
(with 22 figures)

A review of the literature on soil fungi shows that members of the genus *Absidia* have been frequently isolated from soil, and that several species are often present (**1, 2, 4, 9**). In relatively few cases are they apparently absent (**8, 13, 21**). During our studies of the fungi of virgin desert soils in California we have isolated four species of *Absidia* from 390 soils samples obtained in widely separated localities. Three of these species are well known: *Absidia cylindrospora, A. blakesleana* and *A. corymbifera*. In addition to these we have isolated another species eight times from various soils in widely separated areas. Cultural studies show that it is distinct from any of the species previously described. It is described below.

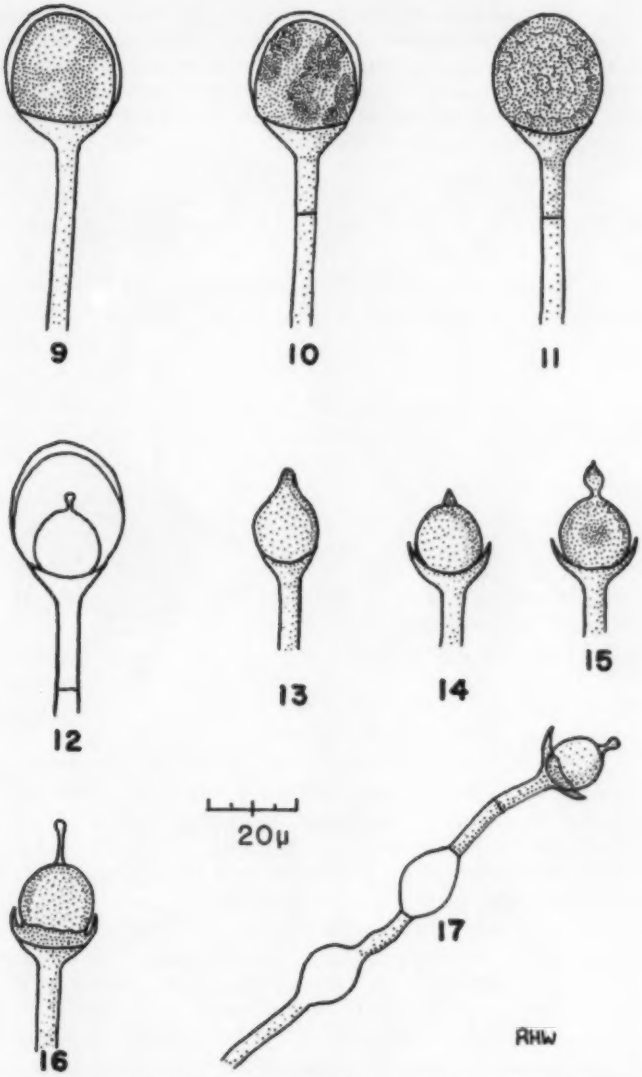
Absidia cuneospora sp. nov.

Coloniae albidae, byssoideae, in maturitate palumbinae, deinde collapsae et intricatae; rhizoidibus hyalinis, pauce ramosis; stolonibus curvatis, hyphis sporangiferis 1–5 ad apices arcus fasciculatis; sporangiis maxima parte globosis 14–40 μ diam. cum apophyso, membrana laevi, hyalina vel fumosa, a limbo facile disjuncta; columella maxima parte hemisphaerica vel globosa et cornu acuto vel obtuso nunquam capitato in apice ornata; sporiis hyalinis, plerumque regulariter cuneatis, 2.0–3.5 × 3.6–7.2 μ; chlamydosporis numerosis, forma et magnitudine variabilibus; cellis giganteis numerosis, sphaericis, 7–22 μ diam.; zygosporis globosis, 50–72 μ diam., rufo-brunneis, delicatule tuberculosis vel reticulatis; appendicibus brunneolis vel rufo-brunneis, irregulariter ramosis, ad unam marginem suspensoris majoris enatis; coitu heterothallico.

Colonies spreading on Synthetic Mucorales Agar (**7**) after 12–14 days at 27° C at first white, later grayish, collapsing, matted; reverse white, somewhat folded; odor none; rhizoids hyaline with few branches; stolons hyaline, curved; sporangiophores to 50 μ in length, mostly arising on arcs of stolons in groups, 1–5 (Figs. 1–3), usually simple or occasionally branched on terminal hyphal filaments (Fig. 1), frequently with vesicular swellings (Fig. 17); sporangia mostly globose (Figs. 9–12), 14–40 μ in diam., apophysate, with a septum 10–18 μ below the apophysis separating the sporangium from the sporangiophore (Figs.

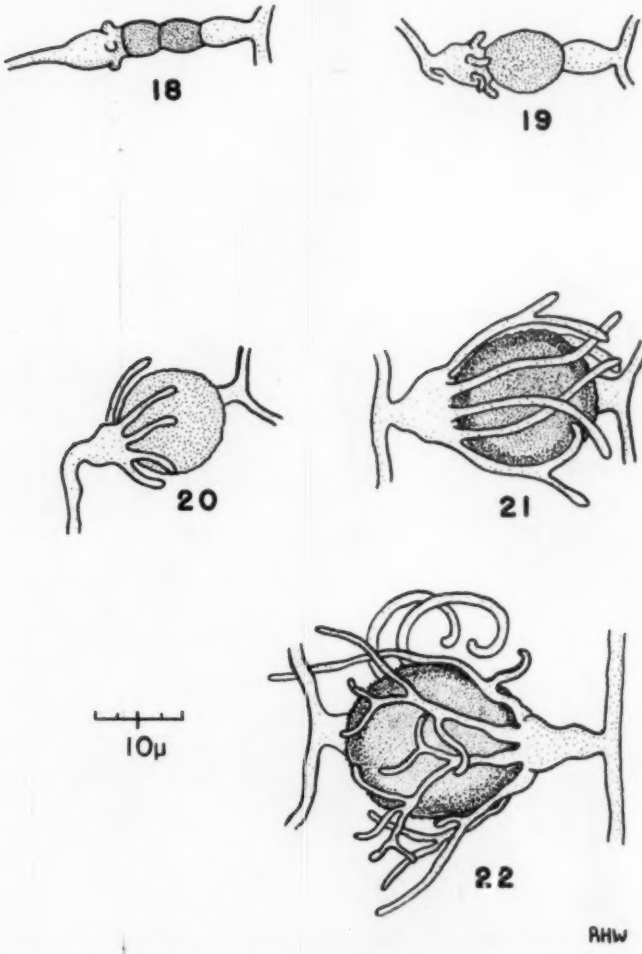


Absidia cuneospora. FIG. 1. Habit. FIGS. 2–3. Attachment of sporangiophores. FIG. 4. Spores. FIGS. 5–7. Chlamydospores. FIG. 8. Giant-cells, free and in the mycelium.



Absidia cuneospora. FIGS. 9–11. Development of sporangia and sporangial septum. FIGS. 12–16. Columellae with collarettes and protuberances. FIG. 17. A vesiculate sporangiophore.

10–12); sporangial walls hyaline to smoky, smooth, breaking apart leaving a collarette (FIGS. 14–17); columellae mostly hemispherical or globose, 14–22 μ in diam., terminating in a blunt, pointed or capitate protuberance (FIGS. 12–17); spores more or less regularly wedge-shaped (FIG. 4), hyaline, 2.0–3.5 μ broad × 3.6–7.2 μ long; chlamydo-



Absidia cuneospora. FIGS. 18–22. Development of zygospore and appendages.

spores numerous, variable in size and shape, present in aerial and substrate mycelium (FIGS. 5–7); giant-cells hyaline, spherical, 7–22 μ in diam. (FIG. 8), present in substrate mycelium in old cultures, becoming free when mycelium disintegrates or is torn; zygospores globose, 50–72 μ in diam., reddish-brown, finely tuberculate or reticulate (FIGS. 21–22); appendages brown to reddish-brown, arising from the larger suspensor, irregularly branched and circinate, somewhat antler-like (FIG. 22); heterothallic.

TYPE-CULTURE (NRRL 2632) from sandy soil, Oildale, California, and the mating type-culture (NRRL 2655) from clay soil, Los Banos, California, deposited at the Northern Utilization Research and Development Division, ARS Culture Collection Investigations, U. S. D. A., Peoria, Ill.

DISCUSSION

Absidia cuneospora is near *A. spinosa* Lendner and *A. cylindrospora* Hagem in the appearance of the sporangia, columellae and general habit. Chlamydospores are more abundant in *A. cuneospora* than in most species of *Absidia*, although one of our isolates of *A. corymbifera* Lichth. exhibits abundant chlamydospores, which are bizarre, and elongate to rounded or irregular in shape. The most distinctive feature of *A. cuneospora* is its wedge-shaped sporangiospore (FIG. 4).

In *A. spinosa* a septum is said to be regularly 25 μ below the apophysis; in *A. cuneospora* the distance of the septum below the apophysis varies from 10–18 μ. This septum is formed just prior to the time of cleavage in the sporangium (FIGS. 9–11).

Colonies of all the isolates were quite similar in appearance. The only differences observed at maturity were in the maximum heights and the less cottony aspect of the turf of some of the isolates.

Zygospores are formed in 8–12 days on SMA agar (7) at 27° where the hyphae of the compatible isolates meet. Gametangia are at first nearly equal, but one of them soon becomes swollen and developes rudimentary appendages quite early (FIG. 18). Gametangia and zygospores of *A. cuneospora* are somewhat smaller than those of *A. cylindrospora* and are difficult to locate in the cottony mass of tangled mycelium. The zygospores of *A. cuneospora* have a finely tuberculate or reticulate exine which is somewhat smoother than that of *A. cylindrospora*. The circinate appendages which arise from the larger suspensor of *Absidia cuneospora* are branched (FIG. 22) while those of *A. spinosa* are simple. The appendages of these two species are considerably shorter than those of *A. cylindrospora*, which are simple and non-circinate. In other species of *Absidia* these appendages may arise from both suspensors.

ACKNOWLEDGMENT

The writers wish to express their grateful thanks to Dr. C. W. Hesseltine for his generous aid in checking the species of *Absidia* and other members of the Mucorales; to Dr. C. C. Epling for his help in preparing the Latin diagnosis; and to R. H. Wishard for the drawings.

SUMMARY

Absidia cuneospora Orr and Plunkett, a new species of *Absidia*, isolated from virgin soils of central and southern California and studied in culture, is herein described and compared to related species. Its most distinctive feature is its wedge-shaped sporangiospore.

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A MONOGRAPH OF THE GENUS UROPYXIS[1]

JOHN W. BAXTER

(WITH 14 FIGURES)

The genus *Uropyxis* was erected by Schroeter (**7**) in 1875, based on *Uropyxis amorphae* (Curt.) Schroet., a rust previously treated as a species of *Puccinia* but differing from other species of that genus in having two pores in each cell of the teliospore. In 1897 the known species of *Uropyxis* were transferred to *Puccinia* by Dietel (**5**). Later the genus *Uropyxis* was reinstated by Magnus (**6**).

The presence of two pores in each cell of the teliospore serves to distinguish *Uropyxis* from *Puccinia*. In addition to this character, *Uropyxis* is now known to possess subcuticular spermagonia and uredinoid aecia. These spermagonial and aecial characters separate it from *Cumminsiella*. *Uropyxis* differs from *Phragmopyxis* in aecial characteristics, in the number of cells in the teliospore and in the number of pores in each teliospore cell.

Of the thirteen known species of *Uropyxis*, ten occur on members of the Leguminosae in North America, South America and Africa; one parasitizes a member of the Cucurbitaceae in Formosa and two occur on Bignoniaceae in South America. The latter two rusts, *Uropyxis rickiana* Magn. and *Uropyxis reticulata* Cumm., differ from other species of the genus in showing no distinct lamination in the teliospore wall. The aecial and spermagonial stages of *U. rickiana* and *U. reticulata* are unknown. In the present study these two species are included in *Uropyxis* with some uncertainty.

In separating species or groups of species in the key, the most useful characters have been found to be the thickness, sculpturing and lamination of the eliospore wall and the length and width of the teliospore pedicel. There is considerable variation throughout the genus with respect to width of the teliospore pedicel, because of the fact that in

[1] Grateful acknowledgment is made to the following: Dr. Sten Ahlner, Naturhistoriska Riksmuseet, Stockholm, Sweden; Dr. G. B. Cummins, Arthur Herbarium, Purdue University; Dr. H. C. Greene, University of Wisconsin, Madison; Dr. Naohide Hiratsuka, Tokyo; Dr. I. Mackenzie Lamb, Farlow Herbarium, Harvard University; and Dr. B. A. Lownens, Department of Agriculture, Pretoria, Union of South Africa.

some species the pedicel is hygroscopic, swelling in water to a characteristic width, while in others it remains narrow, swelling little or not at all.

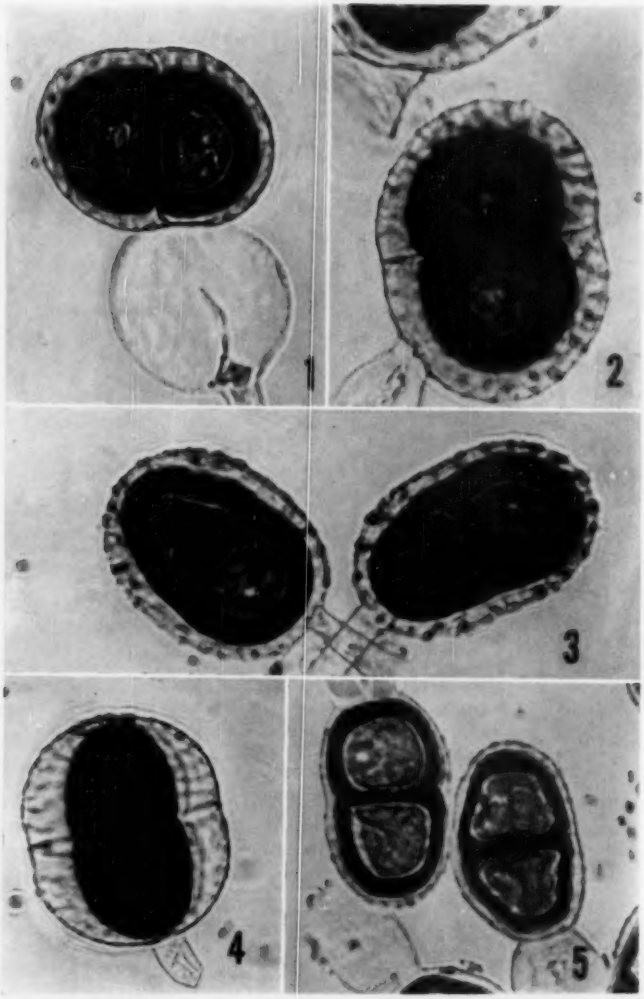
UROPYXIS Schroet., Hedwigia **14**: 165. 1875.

Spermagonia subcuticular, conical, with or without ostiolar paraphyses. Aecia uredinoid, with peripheral paraphyses; aeciospores ellipsoid, obovoid or globoid, wall hyaline or yellowish, echinulate, pores scattered, obscure. Uredia subepidermal, with or without paraphyses; urediospores ellipsoid, obovoid or globoid, wall hyaline or yellowish, echinulate or reticulate, pores scattered or equatorial, usually obscure. Telia subepidermal, with or without paraphyses; teliospores 2-celled, ellipsoid or oblong, wall verrucose-echinulate, minutely verrucose, reticulate or smooth, usually laminate with a brown inner layer and a hygroscopic, hyaline or yellowish outer layer; pores 2 in each cell, opposite; pedicel frequently hygroscopic and swelling in water. Aecial, uredial and telial paraphyses chiefly peripheral, incurved, clavate, capitate or cylindrical, with variable wall thickening.

KEY TO THE SPECIES

Teliospore wall laminate, with a pale outer layer.
- Teliospore wall conspicuously verrucose or echinulate.
  - Teliospore wall 6–15 μ thick.
    - Outer layer of teliospore wall 3–6 μ thick.
      - Pedicel 20–40 μ wide ..... 1. *U. steudneri*
      - Pedicel 10–12 μ wide ..... 2. *U. diphysae*
    - Outer layer of teliospore wall 7–12 μ thick ..... 3. *U. amorphae*
  - Teliospore wall 6 μ or less in thickness.
    - Pedicel 15–20 μ wide ..... 4. *U. daleae*
    - Pedicel 10 μ or less in width.
      - Telia with paraphyses ..... 5. *U. amiciae*
      - Telia lacking paraphyses ..... 6. *U. nissoliae*
- Teliospore wall minutely verrucose, reticulate or smooth.
  - Pedicel 40–160 μ long.
    - Pedicel 30–50 μ wide ..... 7. *U. holwayi*
    - Pedicel 4–5 μ wide ..... 8. *U. wichei*
  - Pedicel less than 40 μ long.
    - Uredia lacking ..... 9. *U. farlowii*
    - Uredia present.
      - Urediospore pores 6–10, scattered ..... 10. *U. petalostemonis*
      - Urediospore pores 2, equatorial ..... 11. *U. arisanensis*

Teliospore wall not conspicuously laminate.
- Teliospore wall chestnut-brown, verrucose ..... 12. *U. reticulata*
- Teliospore wall chocolate-brown, rugose ..... 13. *U. rickiana*



FIGS. 1–5. FIG. 1. Teliospore of *Uropyxis steudneri* (from type). FIG. 2. Teliospore of *U. steudneri* var. *rhodesica* (from type). FIG. 3. Teliospores of *U. diphysae* (from type). FIG. 4. Teliospore of *U. amorphae* (from Solh. Myc. Sax. Exsicc. 79). FIG. 5. Teliospores of *U. dalceae* (from type). All × 800.

1. UROPYXIS STEUDNERI (Magn.) Magn., Ber. Deutsch. Bot. Ges. **10**: 193. 1892. FIG. 1

Diorchidium steudneri Magn., Ber. Deutsch. Bot. Ges. **9**: 91. 1891.
Puccinia steudneri (Magn.) Diet., Jahrb. Wiss. Bot. **26**: 81. 1894.

Spermagonia not seen. Aecia and uredia unknown, probably not formed. Telia amphigenous, mostly hypophyllous, 1–3 mm in diameter, compact, blackish-brown, lacking paraphyses; teliospores broadly ellipsoid, 35–40 × 40–52 μ, rounded at both ends, not or slightly constricted at the septum; wall 6–8 μ thick, verrucose, conspicuously laminate; inner layer 3–4 μ, chestnut-brown, outer layer 3–4 μ, yellow; pedicel hyaline, attached at the septum, inflated to a width of 20–40 μ at the point of attachment, narrowed downward, up to 50 μ long.

HOSTS AND DISTRIBUTION: *Ormocarpum bibracteatum* Baker, Abyssinia.

TYPE SPECIMEN: On *Ormocarpum bibracteatum*, Abyssinia, 1861, *Steudner*.

ILLUSTRATIONS: Sydow, Monog. Ured. **1**: 845, *fig. 562;* this paper (from type).

Uropyxis steudneri is distinct from other members of the genus in having diorchidioid teliospores.

1a. UROPYXIS STEUDNERI Magn. var. RHODESICA Doidge, Bothalia **4**: 917. 1948. FIG. 2

Spermagonia epiphyllous, in groups, flattened-conical, yellow or cinnamon-brown, 105–200 μ in diameter. Telia amphigenous and petiolicolous, 0.5–3.0 mm in diameter, pulverulent or somewhat compact, blackish-brown; teliospores broadly ellipsoid, 35–50 × 44–67 μ, rounded at both ends, not or slightly constricted at the septum; wall 7–10 μ, sparsely and coarsely verrucose-echinulate, with broadly conical tubercles, conspicuously laminate; inner layer 2–4 μ, chestnut-brown, outer layer 5–6 μ, yellowish; pedicel hyaline, usually attached obliquely or at right angles to the septum, occasionally attached at the septum, inflated to a width of 20–25 μ near the point of attachment, narrowed downward, up to 180 μ long.

HOSTS AND DISTRIBUTION: *Ormocarpum trichocarpum* (Taub.) Harmes, Rhodesia.

TYPE SPECIMEN: On *Ormocarpum trichocarpum*, Inyati, Rhodesia, June 26, 1943, *J. C. Hopkins* (Myc. Herb. So. Rhod. 5990).

ILLUSTRATIONS: Doidge, Bothalia **4**: 918, *fig. 19;* this paper (from type).

This variety differs from the typical *U. steudneri* in teliospore size

and pedicel characteristics. There is considerable variation in the point of attachment of the pedicel, which is usually attached obliquely or at right angles to the septum. Relatively few teliospores show the diorchidioid condition characteristic of the typical *U. steudneri*.

2. UROPYXIS DIPHYSAE (Arth.) Cumm., Bull. Torrey Bot. Club **70**: 81. 1943. FIG. 3

Calliospora diphysae Arth., Bot. Gaz. **39**: 391. 1905.

Spermagonia amphigenous, in groups, conical, cinnamon-brown, 100–170 μ in diameter. Telia amphigenous and petiolicolous, 0.5–3 mm in diameter on leaves, 1–5 mm long on petioles, pulverulent, blackish-brown, chocolate-brown or chestnut-brown; teliospores in dark-colored telia ellipsoid, rounded at both ends, not or slightly constricted at the septum, 30–32 × 41–45 μ, wall 6–8 μ thick, verrucose-echinulate, laminate; inner layer 3–4 μ, chestnut-brown, outer layer 3–4 μ, yellow; teliospores in paler telia ellipsoid, rounded at both ends, not or slightly constricted at the septum, 20–29 × 35–40 μ, wall 4.5–5.5 μ thick, verrucose-echinulate, laminate; inner layer about 3 μ, pale chestnut- or cinnamon-brown; outer layer 1.5–2.5 μ, hyaline or pale yellow; pedicel hyaline, with a globoid swelling 10–15 μ in diameter next to the spore or up to 25 μ downward from the point of attachment, remainder of pedicel narrower and usually not inflated, up to 60 μ long.

HOSTS AND DISTRIBUTION: *Diphysa robinoides* Benth., Guatemala, Costa Rica; *Diphysa suberosa* Wats., Mexico; *Diphysa* sp., Guatemala, Costa Rica.

TYPE SPECIMEN: On *Diphysa suberosa*, Rio Blanco, Guadalajara, Mexico, Sept. 30, 1903, *Holway 5082*.

ILLUSTRATIONS: This paper (from type).

EXSICCATI: Barth. N. Am. Ured. 208 (as *Calliospora diphysae*).

This rust was first described by Arthur (**1**) as *Calliospora diphysae*. The genus *Calliospora* was established by Arthur to accommodate several species having subcuticular spermagonia and two-celled teliospores with two pores in each cell, but lacking aecia and uredia. Such rusts are now treated as microcyclic species of *Uropyxis*.

3. UROPYXIS AMORPHAE (Curt.) Schroet., Hedwigia **14**: 165. 1875. FIG. 4

Puccinia amorphae Curt., Am. Jour. Sci. II. **6**: 353. 1848.
Aecidium amorphae Cooke, Grevillea **6**: 137. 1878.
Uredo kansensis Kell. & Sw., Jour. Myc. **5**: 77. 1889.

Spermagonia amphigenous, mostly epiphyllous, in groups, conical, cinnamon-brown or yellowish, 60–90 μ in diameter. Aecia hypophyllous,

in groups, uredinoid, with peripheral paraphyses; aeciospores ellipsoid, 14–23 × 20–33 μ; wall 1.5–3 μ thick, hyaline, echinulate, pores 8–12, scattered, obscure. Uredia amphigenous, chiefly epiphyllous, 0.2–0.5 mm in diameter, scattered or in circular groups, pulverulent, yellow or pale cinnamon-brown, with peripheral paraphyses; urediospores ellipsoid or obovoid, 14–20 × 18–30 μ, wall 2–2.5 μ, yellowish, echinulate, pores 10–12, scattered, obscure. Telia amphigenous and caulicolous, 0.3–2 mm in diameter, pulverulent or somewhat compact, blackish-brown, with peripheral paraphyses; teliospores broadly ellipsoid, 30–36 × 35–48 μ, rounded at both ends, slightly constricted at the septum; wall 9–15 μ thick, verrucose, laminate; inner layer 2–3 μ, chocolate-brown, outer layer 7–12 μ at sides, 1–3 μ at base and apex, hyaline; pedicel hyaline, lower portion swelling in water and disappearing, upper portion not swelling, frequently breaking away at the point of attachment, length of spore or shorter.

HOSTS AND DISTRIBUTION: *Amorpha angustifolia* (Pursh) Boyn., *A. californica* Nutt., *A. canescens* Pursh, *A. fragrans* Sweet, *A. fruticosa* L., *A. herbacea* Walt., *A. montana* Boyn., *A. nana* Nutt., *A. tennesseensis* Shuttlew., *A. virgata* Small, *Amorpha* spp., central, southern and western United States; *Parryella filifolia* Torr. & Gray, New Mexico.

TYPE SPECIMEN: On *Amorpha herbacea*, Society Hill, S. Carolina, *Ravenel*.

ILLUSTRATIONS: Arthur, Manual of Rusts, p. 77, *fig. 99;* this paper (from Solheim Myc. Sax. Exsicc. 79).

EXSICCATI: Barth. N. Am. Ured. 99, 200, 1399, 2300, 2400, 2600, 2896, 2897, 3198, 3298, 3299; Barth., Ell. & Ev. Fungi Columb. 1690, 1691; Barth. Fungi Columb. 2496, 2698, 3598, 3997, 4097; Brenckle N. Dak. Fungi 324, 333, 334, 489, 490, 596; Brenckle Fungi Dak. 88, 192, 248; Ell. & Ev. N. Am. Fungi 2418, 3148; Kell & Sw. Kansas Fungi 905; Ravenel Fungi Am. Exsicc. 39, 40, 279; Solheim Myc. Sax. Exsicc. 79, 286, 345, 600; Syd. Ured. 1034, 1035, 2509.

Among species of *Uropyxis, U. amorphae* exhibits the most marked development of the hygroscopic outer layer in the teliospore wall. When the teliospores are viewed dry the wall appears to be composed of one layer, only the inner dark-colored layer being evident. The bilaminate nature of the wall becomes apparent after the spores are mounted in liquid. In a water mount the outer layer swells rapidly, frequently bursting, and is seen to be composed of a thick gelatinous layer overlaid by a thin verrucose cuticle. The cuticle frequently splits and peels away from the underlying layer, which then may dissolve.

4. UROPYXIS DALEAE (Diet. & Holw.) Magn., Ber. Deutsch. Bot. Ges. **17**: 115. 1899. FIG. 5

Puccinia daleae Diet. & Holw., Bot. Gaz. **24**: 27. 1897.

Spermagonia and aecia unknown. Uredia hypophyllous, 0.5–1.0 mm in diameter, scattered or in circular groups on yellow spots, pulverulent, cinnamon-brown, with peripheral paraphyses; urediospores broadly ellipsoid, 16–22 × 18–25 μ, wall 1–1.5 μ, yellowish or hyaline, echinulate, pores 6–10, scattered, obscure. Telia hypophyllous, 0.5–1.0 mm in diameter, pulverulent, blackish-brown, with peripheral paraphyses; teliospores ellipsoid, 24–30 × 35–50 μ, rounded at both ends, not or slightly constricted at the septum; wall 4–6 μ, verrucose-echinulate, laminate; inner layer 2.5–4 μ, chestnut or dark cinnamon-brown; outer layer 1.5–2 μ, yellowish; pedicel hyaline, swelling in water to a width of 15–20 μ, up to 35 μ long.

HOSTS AND DISTRIBUTION: *Dalea alopecuroides* Willd., Argentina; *Parosela acutifolia* (DC.) Rose, Mexico; *Parosela citriodora* (Willd.) Rose, Mexico; *P. dalea* (L.) Britt., Mexico; *P. diffusa* (Moric.) Rose, Guatemala; *P. domingensis* (DC.) Millsp., Guatemala; *P. holwayi* Rose, Mexico; *P. mucronata* Rose, Mexico; *P. mutabilis* (Cav.) Rose, Mexico; *P. nutans* (Cav.) Rose, Guatemala, El Salvador; *P. pazensis* Rusby, Bolivia; *P. trifoliata* (Zucc.) Rose, Mexico.

TYPE SPECIMEN: On *Parosela mutabilis*, Tula, Mexico, Oct. 5, 1896, *Holway*.

ILLUSTRATIONS: This paper (from type).

EXSICCATI: Barth. N. Am. Ured. 1298; Holw. Plants of S. Am. 333; Rel. Holw. 245; Syd. Ured. 1519; Vestergren Micro. Rar. Sel. 1508.

The chief distinguishing character in *Uropyxis daleae* is the teliospore pedicel, which swells to a width of 15–20 μ at the point of attachment to the spore, breaking or dissolving at a point 15 to 30 μ from the spore and only rarely retaining the narrowed lower portion.

4a. UROPYXIS DALEAE (Diet. & Holw.) Magn. var. **eysenhardtiae** (Diet. & Holw.) stat. nov.

Puccinia eysenhardtiae Diet. & Holw., Bot. Gaz. **24**: 27. 1897.

Uropyxis eysenhardtiae (Diet. & Holw.) Magn., Ber. Deutsch. Bot. Ges. **17**: 115. 1899.

Spermagonia and aecia unknown. Uredia hypophyllous, 0.2–0.5 mm in diameter, pulverulent, cinnamon-brown, with peripheral paraphyses; urediospores globoid or ellipsoid, 14–17 × 15–21 μ, wall about 1 μ, yellowish, echinulate, pores 6–10, scattered, obscure. Telia hypophyllous, scattered, 0.2–1 mm in diameter, pulverulent, chocolate-brown, with

peripheral paraphyses; teliospores ellipsoid, 22–30 × 35–44 μ, rounded at both ends, slightly or not constricted at the septum; wall 4–6 μ, sparsely and evenly verrucose-echinulate, laminate; inner layer 2–3 μ, chestnut-brown; outer layer 2–3 μ, yellowish; pedicel hyaline, swelling in water to a width of 15–20 μ, up to 30 μ long.

HOSTS AND DISTRIBUTION: *Dalea albiflora* Gray, Arizona; *Eysenhardtia amorphoides* H.B.K., Mexico; *Eysenhardtia orthocarpa* (Gray) Wats., Arizona, Mexico.

TYPE SPECIMEN: On *Eysenhardtia orthocarpa*, near Mexico City, Mexico, Oct. 1, 1896, *Holway*.

Uropyxis eysenhardtiae differs from *U. daleae* only in having slightly smaller urediospores and teliospores. It seems advisable to reduce this species to varietal rank within *U. daleae*.

4b. UROPYXIS DALEAE (Diet. & Holw.) Magn. var. **africana** var. nov.

A typo differt teliosporis longioribus, telia plerumque epiphylla, 0.2–0.5 mm diam.

Spermagonia, aecia and uredia unknown. Telia amphigenous, mostly epiphyllous, 0.2–0.5 mm in diameter, pulverulent, blackish-brown, with peripheral paraphyses; teliospores ellipsoid or oblong, 21–30 × 36–52 μ, rounded at both ends, not or slightly constricted at the septum; wall 4–6 μ, sparsely and evenly verrucose-echinulate, laminate; inner layer 2–3 μ, chestnut-brown, outer layer 2–3 μ, hyaline; pedicel hyaline, swelling in water to a width of 15–17 μ, 20 μ or less in length.

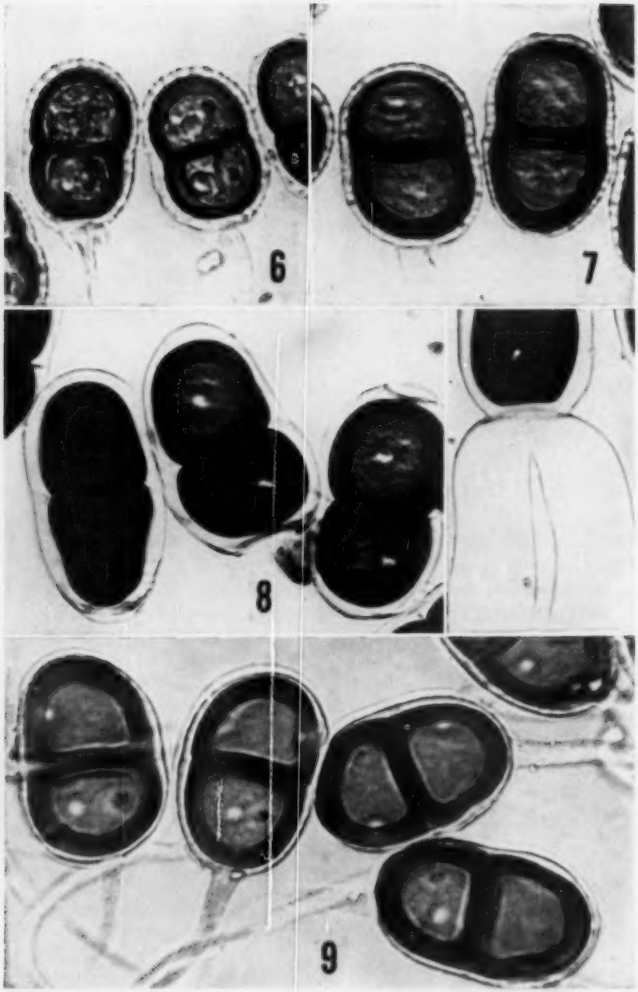
HOSTS AND DISTRIBUTION: *Aeschynomene trigonocarpa* Taub., Rhodesia.

TYPE SPECIMEN: On *Aeschynomene trigonocarpa*, Mt. Makulu, near Lusaka, Northern Rhodesia, May 20, 1956, *Angus M.143* (Herb. I.M.I. No. 70515).

This variety differs from the typical *U. daleae* in having slightly longer teliospores and in forming smaller, chiefly epiphyllous telia. I am indebted to Dr. F. C. Deighton, Commonwealth Mycological Institute, and Dr. G. B. Cummins, Purdue University, for the opportunity to examine the type material.

5. UROPYXIS AMICIAE Jacks. & Holw. in Jacks., Mycologia **23**: 356. 1931. FIG. 6

Spermagonia and aecia unknown. Uredia amphigenous, 0.3–1.0 mm in diameter, pulverulent, pale cinnamon-brown, with peripheral paraphyses; urediospores globoid or broadly ellipsoid, 15–19 × 16–20 μ, wall



FIGS. 6–9. FIG. 6. Teliospores of *U. amiciae* (from neotype). FIG. 7. Teliospores of *U. nissoliae* (from type). FIG. 8. Teliospores of *U. holwayi* (from type). FIG. 9. Teliospores of *U. wichei* (from type). All × 800.

1.5–2.5 μ thick, yellowish or hyaline, echinulate, pores 8–12, scattered, obscure. Telia amphigenous, 0.5–1 mm in diameter, pulverulent, blackish-brown, with peripheral paraphyses; teliospores ellipsoid, 18–20 × 28–34 μ, rounded at both ends, not or slightly constricted at the septum; wall 4–6 μ thick, finely verrucose-echinulate, laminate; inner layer 2–3 μ thick, chestnut-brown; outer layer 2–3 μ thick, hyaline; pedicel hyaline, not swelling appreciably, length of spore or shorter.

HOSTS AND DISTRIBUTION: *Amicia parvula* Rusby, Bolivia.

TYPE SPECIMEN: On *Amicia parvula*, Cochabamba, Bolivia, March 7, 1920, *Holway* (NEOTYPE).

ILLUSTRATIONS: This paper (from neotype).

EXSICCATI: Rel. Holw. 244.

The name *Puccinia amiciae* Vestergr. is a *nomen nudum*. Apparently a type specimen was not clearly designated by Vestergren. In view of this, the Holway specimen from Bolivia is here designated the neotype.

6. UROPYXIS NISSOLIAE (Diet. & Holw.) Magn., Ber. Deutsch. Bot. Ges. **17**: 115. 1899. FIG. 7

Puccinia nissoliae Diet. & Holw., Bot. Gaz. **24**: 27. 1897.

Spermagonia epiphyllous, conical, cinnamon-brown, 75–120 μ in diameter. Aecia hypophyllous, in groups, uredinoid, with peripheral paraphyses; aeciospores globoid or ellipsoid, 12–17 × 14–20 μ; wall 1 μ thick, hyaline or yellowish, closely echinulate, pores 6–8, scattered, obscure. Uredia hypophyllous, 0.1–0.2 mm in diameter, pulverulent, cinnamon-brown, with peripheral paraphyses; urediospores globoid or ellipsoid, 12–15 × 15–20 μ, wall about 1 μ thick, hyaline or yellowish, pores 6–8, scattered, obscure. Telia amphigenous, 0.2–1.0 mm in diameter, pulverulent, chocolate-brown, lacking paraphyses; teliospores ellipsoid, 20–27 × 30–40 μ, rounded at both ends, slightly constricted at the septum; wall 4–6 μ, verrucose-echinulate, laminate; inner layer 2.5–4 μ, chestnut-brown; outer layer 1.5–2 μ, yellowish or hyaline; pedicel hyaline, usually roughened, not swelling in water, length of spore or shorter.

HOSTS AND DISTRIBUTION: *Nissolia confertiflora* Wats., Mexico; *N. fruticosa* Jacq., El Salvador, Guatemala; *N. hirsuta* DC., *N. laxior* Rose, *N. multiflora* Rose, Mexico; *Nissolia* spp., Mexico.

TYPE SPECIMEN: On *Nissolia confertiflora*, Guadalajara, Mexico, Oct. 12, 1896, *Holway*.

ILLUSTRATIONS: This paper (from type).

EXSICCATI: Barth. N. Am. Ured. 1299, Syd. Ured. 1524.

This species resembles *U. amiciae*, differing chiefly in having larger teliospores and in the absence of paraphyses in the telia.

7. UROPYXIS HOLWAYI (Arth.) Arth., Man. Rusts U. S. and Canada 77. 1934. FIG. 8

Calliospora holwayi Arth., Bot. Gaz. **39**: 390. 1905.

Spermagonia epiphyllous, conical, cinnamon-brown or yellowish, 70–140 μ in diameter. Telia epiphyllous, 0.5–2 mm in diameter, pulverulent, chestnut-, chocolate- or blackish-brown, lacking paraphyses; teliospores in paler telia ellipsoid, rounded at both ends, slightly constricted at the septum, 19–24 × 35–51 μ, wall 2–3.5 μ, smooth, laminate; inner layer 1–2 μ, cinnamon-brown; outer layer 1–1.5 μ, hyaline; teliospores in darker telia ellipsoid, rounded at both ends, slightly constricted at the septum, 24–29 × 39–53 μ, wall 4–7 μ, smooth, laminate, inner layer 2–3 μ, chocolate-brown; outer layer 2–4 μ, hyaline; pedicel hyaline, swelling in water to a width of 30–50 μ, 40–60 μ long.

HOSTS AND DISTRIBUTION: *Diphysa floribunda* Peyr., Guatemala; *Eysenhardtia adenostylis* Baill., Guatemala; *Eysenhardtia amorphoides* H.B.K., Mexico; *Eysenhardtia orthocarpa* (Gray) Wats., Mexico, Arizona.

TYPE SPECIMEN: On *Eysenhardtia orthocarpa*, Etla, Oaxaca, Mexico, Nov. 13, 1903, *Holway*.

ILLUSTRATIONS: This paper (from type).

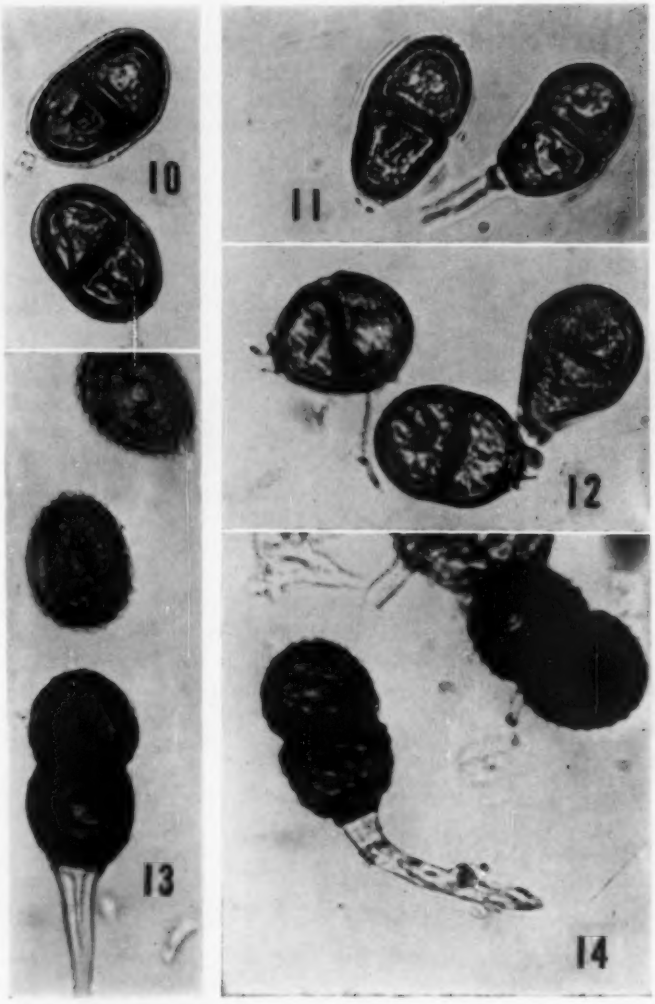
EXSICCATI: Barth. N. Am. Ured. 2.

The smooth wall of the teliospore and the width of the pedicel serve to separate this rust from the other microcyclic species of *Uropyxis*. As in *U. diphysae*, there is considerable variability in size and color of teliospores and in color of telia.

8. UROPYXIS WIEHEI Cumm., Bull. Torrey Bot. Club **83**: 232. 1956. FIG. 9

Spermagonia and aecia unknown. Uredia uncertain, probably caulicolous, with peripheral paraphyses, urediospores broadly ellipsoid or globoid, 17–20 × 20–25 μ, wall 2.5–3 μ thick, yellowish or hyaline, echinulate, pores 6–8, scattered, obscure. Telia caulicolous, causing slight hypertrophy, pulverulent or somewhat compact, confluent, dark cinnamon-brown or chestnut-brown; teliospores ellipsoid, 27–33 × 38–48 μ, rounded at both ends, not constricted at the septum; wall 4–7 μ thick, smooth or minutely verrucose-echinulate, laminate; inner layer 3.5–5 μ, chestnut- or dark cinnamon-brown; outer layer 0.5–2 μ, hyaline; pedicel hyaline, not swelling in water, persistent, up to 160 μ long.

HOSTS AND DISTRIBUTION: *Aeschynomene abyssinica* Vatke, Nyasaland; *Aeschynomene trigonocarpa* Taub., Northern Rhodesia.



FIGS. 10–14. FIG. 10. Teliospores of *U. farlowii* (from type). FIG. 11. Teliospores of *U. petalostemonis* (from type). FIG. 12. Teliospores of *U. arisanensis* (from type). FIG. 13. Teliospore and urediospores of *U. reticulata* (from type). FIG. 14. Teliospores of *U. rickiana* (from type). All × 800.

TYPE SPECIMEN: On *Aeschynomene abyssinica*, Dedza, Nyasaland, Sept. 4, 1950, *P. O. Wiehe 861*.

ILLUSTRATIONS: Cummins, Bull. Torrey Bot. Club **83**: 230, *fig. 15;* this paper (from type).

The length of the teliospore pedicel serves to distinguish this rust from other species of *Uropyxis*.

9. **Uropyxis farlowii** (Arth.) comb. nov. FIG. 10

Calliospora farlowii Arth., Bot. Gaz. **39**: 39. 1905.

Spermagonia caulicolous and amphigenous, conical, cinnamon-brown or yellowish, 80–100 μ in diameter. Telia caulicolous, 2–3 mm in diamter, in groups 6–16 mm long, causing slight hypertrophy, pulverulent, chestnut-brown, lacking paraphyses; teliospores ellipsoid, 18–24 × 28–40 μ, rounded at both ends, not or slightly constricted at the septum, wall 3–4.5 μ, minutely verrucose, laminate; inner layer 2–3 μ, cinnamon-brown; outer layer 1–1.5 μ, hyaline; pedicel hyaline, not swelling noticeably in water, length of spore or shorter.

HOSTS AND DISTRIBUTION: *Parosela domingensis* (DC.) Heller, Mexico, Cuba.

TYPE SPECIMEN: On *Parosela domingensis*, Orizaba, Mexico, Feb., 1885, *Farlow*.

ILLUSTRATIONS: This paper (from type).

Uropyxis farlowii was first described by Arthur (**1**) as a species of *Calliospora*. As was pointed out by Cummins and Stevenson (**4**), the need for the transfer of this species to *Uropyxis* has been overlooked.

10. UROPYXIS PETALOSTEMONIS (Farl.) DeT., in Sacc. Syll. Fung. **7**: 735. 1888. FIG. 11

Puccinia petalostemonis Farl., in Trelease, Trans. Wis. Acad. **6**: 129. 1884.

Calliospora petalostemonis Arth., Bull. Torrey Bot. Club **34**: 588. 1908.

Uropyxis affinis Arth., Man. Rusts U. S. and Canada 76. 1934.

Spermagonia hypophyllous, frequently evenly scattered and appearing systemic, conical, cinnamon-brown or yellowish, 80–130 μ in diameter. Aecia hypophyllous, in groups or evenly scattered with a systemic appearance, uredinoid, with peripheral paraphyses; aeciospores ellipsoid or obovoid, 16–21 × 20–27 μ, wall 1.5–2 μ thick, hyaline, echinulate, pores 6–10, scattered, obscure. Uredia hypophyllous, 0.2–0.5 mm in diameter, pulverulent, cinnamon-brown, with peripheral paraphyses;

urediospores ellipsoid, obovoid or globoid, 18–21 × 20–26 μ, wall 1.5–2 μ, yellowish, echinulate, pores 6–10, scattered, obscure. Telia hypophyllous, 0.2–1.0 mm in diameter, pulverulent, chocolate- or blackish-brown, with peripheral paraphyses; teliospores ellipsoid, 21–30 × 38–45 μ, rounded at both ends, slightly constricted at the septum; wall 3–5 μ thick, finely verrucose, laminate; inner layer 2–3 μ, chestnut-brown; outer layer 1–2 μ, yellowish; pedicel hyaline, not swelling in water, 10–35 μ long.

HOSTS AND DISTRIBUTION: *Petalostemon candidus* (Willd.) Michx., *P. compactus* (Spreng.) Sw., *P. multiflorus* Nutt., *P. oligophyllus* (Torr.) Rydb., *P. purpureus* (Vent.) Rydb., *Petalostemon* sp., western United States, western Canada.

TYPE SPECIMEN: On *Petalostemon* sp., LaCrosse, Wisconsin, July, 1883, *Pammel.*

ILLUSTRATIONS: Arthur, Man. Rusts, p. 76, *fig. 98;* this paper (from type).

EXSICCATI: Barth. Fungi Columb. 4699, 4896; Barth. N. Am. Ured. 400, 900, 1200, 1799, 2898, 2899, 3300; Brenckle Fungi Dak. 89, N. Dak. Fungi 299; Carleton Ured. Am. 8; Clements Crypt. Form. Colo. 583; Standley Fungi of N. M. 4960.

Examination of a large number of specimens of *U. petalostemonis* during the present study has revealed that this species is unstable as to life cycle. Certain specimens bear spermagonia, aecia and telia, with apparent suppression of the uredial stage, while other specimens lack the aecial stage. One collection, on *Petalostemon oligophyllus* from Saskatchewan, was found to consist of spermagonia and telia only, with a few urediospores in the telia. The type of *U. affinis,* also on *P. oligophyllus* from New Mexico, was found to be similar to the Saskatchewan material. *U. affinis* was first described by Arthur (**2**) as *Calliospora petalostemonis.* Arthur described this species as forming only spermagonia and telia, the latter lacking paraphyses. I have found a few urediospores and paraphyses in telia of the type material. In accord with these findings and in view of the life-cycle variability shown by *U. petalostemonis,* it seems advisable to reduce *U. affinis* to synonymy.

11. UROPYXIS ARISANENSIS (Hirats. & Hash.) S. Ito & Murayama, Mem. Tott. Agr. Coll. **7**: 34. 1943. FIG. 12

Puccinia arisanensis Hirats. & Hash. in Hiratsuka, Bot. Mag. Tokyo **55**: 270. 1941.

Spermagonia and aecia unknown. Uredia hypophyllous, scattered, 0.2–0.5 mm in diameter, pulverulent, cinnamon- or chestnut-brown;

urediospores broadly ellipsoid or globoid, 16–20 × 23–30 μ, wall 1–1.5 μ, cinnamon-brown, echinulate, pores 2, equatorial. Telia hypophyllous, scattered, 0.1–0.5 mm in diameter, pulverulent, chestnut-, chocolate- or blackish-brown; teliospores ellipsoid, 20–25 × 30–40 μ, rounded at the apex, rounded or somewhat narrowed at the base, not constricted at the septum; wall 2–3 μ, finely reticulate-verrucose, laminate; inner layer cinnamon- or chestnut-brown, outer layer yellowish or hyaline; pedicel hyaline, length of spore or shorter.

HOSTS AND DISTRIBUTION: *Melothria mucronata* Cogn., Formosa.

TYPE SPECIMEN: On *Melothria mucronata,* Prov. Tainan, Funkiko (Mt. Arisan), Formosa, Jan. 16, 1941, *Hiratsuka and Hashioka.*

ILLUSTRATIONS: Ito, Mycol. Flora of Japan **2**: 19, *fig. 20;* this paper (from type).

Uropyxis arisanensis is the only species of *Uropyxis* known to parasitize Cucurbitaceae. The reticulate sculpturing of the teliospore wall and the presence of two equatorial pores in the urediospore are the distinctive features of this species.

12. UROPYXIS RETICULATA Cumm., Mycologia **31**: 171. 1939. FIG. 13

Spermagonia and aecia unknown. Uredia hypophyllous and caulicolous, 0.5–2.0 mm in diameter, cinnamon- or chestnut-brown, pulverulent; urediospores broadly ellipsoid or obovoid, 16–22 × 23–27 μ, wall 2 μ thick, cinnamon-brown, reticulate, pores 2, equatorial. Telia caulicolous, confluent on hypertrophied stems, chestnut-brown, pulverulent; teliospores ellipsoid or oblong, 18–23 × 31–40 μ, rounded at both ends, slightly to sharply constricted at the septum; wall 2.5–3 μ, verrucose, chestnut- or dark cinnamon-brown; pedicel hyaline, persistent, frequently somewhat swollen and roughened in the lower third, 30–65 μ long.

HOSTS AND DISTRIBUTION: *Arrabidea* sp., Paraguay; *Bignonia unguis-cati* L., Argentina.

TYPE SPECIMEN: On *Bignonia unguis-cati,* LaPlata, Argentina, Feb. 1937, *Lindquist.*

ILLUSTRATIONS: Cummins, Mycologia **31**: 172, *fig. 4;* this paper (from type).

U. reticulata is distinguished from the other species of the genus by the reticulate sculpturing of the urediospore wall. It also differs from most other species of *Uropyxis* in showing no distinct lamination in the teliospore wall.

13. UROPYXIS RICKIANA Magn., Hedwigia **45**: 176. 1906. FIG. 14

Spermagonia, aecia and uredia unknown. Telia caulicolous, causing hypertrophy, compact, blackish-brown, confluent, occurring on or beneath the surface of corky galls up to 6 cm in diameter; teliospores ellipsoid or oblong, 20–26 × 33–43 μ, rounded at both ends, slightly to sharply constricted at the septum; wall 2.5–3(–4) μ, rugose, chocolate-brown or olivaceous; pedicel hyaline, 30–80 μ long.

HOSTS AND DISTRIBUTION: Bignoniaceae (unidentified), Brazil.

TYPE SPECIMEN: On Bignoniaceae (unidentified), São Leopoldo, Brazil, 1905, *Rick*.

ILLUSTRATIONS: Magnus, Hedwigia **45**: *pl. 9, figs. 1–10;* this paper (from type).

EXSICCATI: Rick Fungi Austro-Americani 105.

This species closely resembles *U. reticulata*, differing only in the color and sculpturing of the teliospore wall. Both species occur on members of the Bignoniaceae and cause hypertrophy of the host. Possibly *U. rickiana* and *U. reticulata* should be treated as varieties of a single species but since urediospores have not been described for *U. rickiana* the two taxa are here treated as separate species.

EXCLUDED AND DOUBTFUL SPECIES

1. UROPYXIS FRAXINI (Kom.) Magn., Ber. Deutsch. Bot. Ges. **17**: 117. 1899.

Puccinia fraxini Kom., in Jacz., Kom. and Tranzschel Fungi Rossi Exsicc.: 225. 1898.

The presence of three (occasionally four) pores in each cell of the teliospore excludes this species from *Uropyxis*. Possibly it should be placed in the genus *Cleptomyces*.

2. UROPYXIS ROSEANA Arth., N. Am. Flora **7**: 157. 1907.

No teliospores could be found in the scanty type material of this species.

3. UROPYXIS GERSTNERI Doidge, Bothalia **4**: 916–917. 1948.

Examination of the type material has revealed that there is a single pore in each cell of the teliospore. This character and the presence of a vertical septum place this species in the genus *Diorchidium*.

4. UROPYXIS ANTARCTICA (Speg.) Lindquist, Notas Museo LaPlata **11**: 372. 1947.

Puccinia antarctica Speg., Bol. Acad. Cienc. Cord. **11**: 179. 1887.
Uropyxis naumanniana Magn., Ber. Deutsch. Bot. Ges. **10**: 320. 1892.
Puccinia naumanniana (Magn.) Neger, Ann. Univ. Chile **93**: 787. 1896.
Puccinia magellanica Speg., Rev. Chil. Hist. Nat. **25**: 267. 1921.

5. UROPYXIS STOLPIANA (Diet. and Neg.) Magn., Ber. Deutsch. Bot. Ges. **27**: 120. 1899.

Uredo stolpiana Magn., Ber. Deutsch. Bot. Ges. **11**: 325. 1892.
Puccinia stolpiana Diet. and Neg., Bot. Jahrb. **27**: 13. 1899.

Both *Uropyxis antarctica* and *U. stolpiana* were recently transferred by Baxter (**3**) to the genus *Cumminsiella*.

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FUNGUS NUCLEI IN THE DIAGNOSIS OF MYCOSES

CHESTER W. EMMONS

(WITH 20 FIGURES)

The nuclei of fungi which cause human disease are too small and delicate to be seen clearly, if at all, in sections of infected tissues prepared by routine histologic technics in the clinical laboratory. Although such preparations may be adequate for diagnostic purposes and for interpretations of host-cell reactions, the morphologic integrity of the fungus nucleus is lost and cytoplasmic structures are distorted or destroyed by shrinkage or other forces. These finer details of fungus cell structure may be of considerable practical diagnostic importance. If no culture has been isolated, information about the number and appearance of nuclei may aid in the differential identification of such fungi as *Histoplasma* and *Blastomyces*.

These practical considerations and the paucity of adequate and accurate illustrations of fungus nuclei in mycoses appear to justify the publication of a paper illustrating these nuclei and describing methods whereby they can be demonstrated. Early descriptions of some of the pathogens are illustrated by photographs or drawings in which intracellular granules or deeply staining bodies are shown. Some of these may represent concentrations of nuclear material, but they do not show the characteristic morphology of the fungus nucleus, probably because of unsuitable fixation or the rapidity with which dehydration and paraffin infiltration were carried out.

MATERIALS AND METHODS

Infected human tissues have not been readily available for most of these studies. Therefore, I have used tissues from experimentally infected animals. Mice were infected by the intravenous injection of yeast cells of *Cryptococcus neoformans*, *Histoplasma capsulatum* or *Blastomyces dermatitidis*, and spleen, liver and lung were subsequently removed and processed. The nuclei of *B. dermatitidis* are illustrated also in photomicrographs of sections from a human dermal lesion which I prepared in 1931 and have used in teaching but have not previously

published. The photomicrographs of *Coccidioides immitis* show the fungus in lesions in the abdominal wall of a guinea pig. Photomicrographs of sections of this same lesion were used to illustrate my paper on coccidioidomycosis published in 1942 in MYCOLOGIA. *Paracoccidioides brasiliensis* is shown in metastic lesions in a hamster.

All tissues were fixed in a modification of Bouin's fixative, Wilson's B-15,[1] which the writer was taught to use by the late Prof. Robert A. Harper. Tissues were kept in fixative 10 to 20 hours and washed in 70 percent alcohol. They were then dehydrated over a period of 2 to 4 hours by changes to increasingly concentrated alcohol; the alcohol was replaced by four changes of graded mixtures of alcohol and xylol, they were cleared in xylol, with paraffin added were held at room temperature for 2 to 5 days, and finally the infiltration was completed with paraffin in the paraffin oven.

Sections were stained with Heidenhain's iron-alum haematoxylin, using Yamanouchi's schedule, as given in Chamberlain's "Methods in Plant Histology" (**4**), p. 45. The nuclear nature of the structure, assumed on the basis of form to be a nucleus, was confirmed by Feulgen stains. Sections, stains and photomicrographs are by the writer. Negatives were developed by Wm. A. Hill and prints were made in the Photographic Section, N.I.H.

RESULTS

The fungus nucleus, as it has been illustrated for many nonpathogenic fungi, consists of a very delicate nuclear membrane, a poorly stained nucleoplasm and a single spherical densely stained nucleolus. As in all histologic preparations, there is room for interpretation of how accurately the artefacts produced by the technics represent actual morphologic structures in the living cell. The uniformity with which the tiny nucleus is preserved and the lack of apparent distortion indicate that the above method of preparation gives a reliable representation of the nucleus.

Coccidioides

Some of the early descriptions of *Coccidioides,* such as those of Wolbach (**10**) and Ahlfeldt (**1**), are illustrated by photographs or

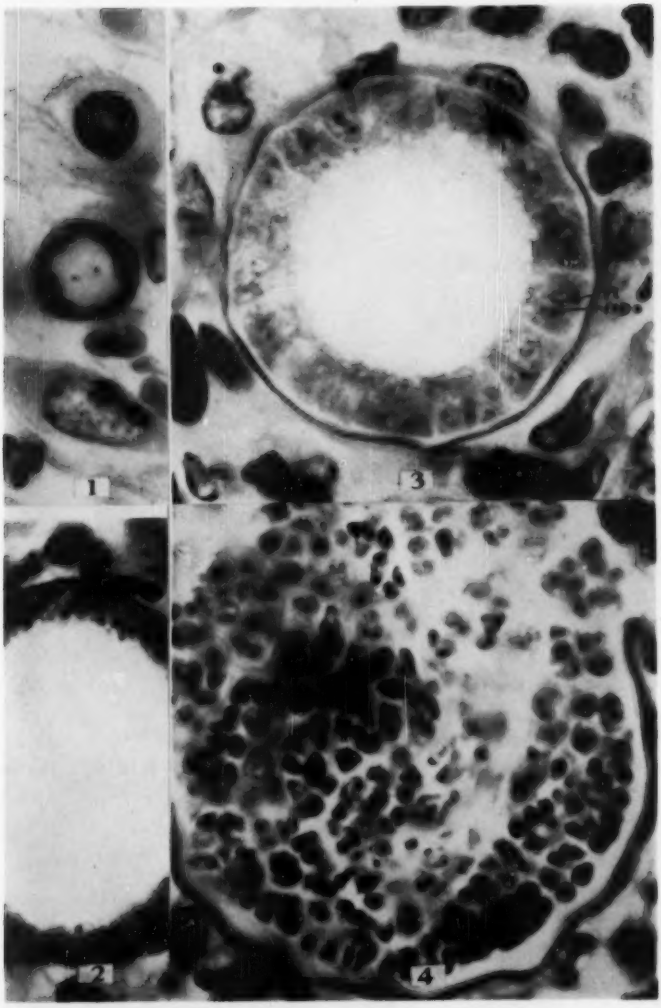
[1] *Solution A*

Saturated aqueous picric acid	190 cc
Glacial acetic acid	20 cc
Chromic acid	4 g

Solution B

Saturated aqueous picric acid	110 cc
Formalin	100 cc
Urea crystals	4 g

Mix equal parts of A and B just before use.



FIGS. 1–4. *Coccidioides immitis*. 1. Young spherules. 2. Spherule prior to sporulation. 3. Multinucleate protospores. 4. Uninucleate sporangiospores. n—nucleus, no—nucleolus. × 1600.

drawings in which densely stained areas in the fungus cell may be interpreted as representing nuclear material. In none of these early papers, however, is there a clear description or an acceptable illustration of the nucleus. The nucleus of *Coccidioides* was first illustrated unequivocally in my paper on coccidioidomycosis in 1942 and the nuclei are most clearly shown there (**8**) in *Fig. 4, 6 and 7*. In the paper by Baker, Mrak and Smith, published in 1943 (**2**), the multinucleate condition of *Coccidioides* was confirmed.

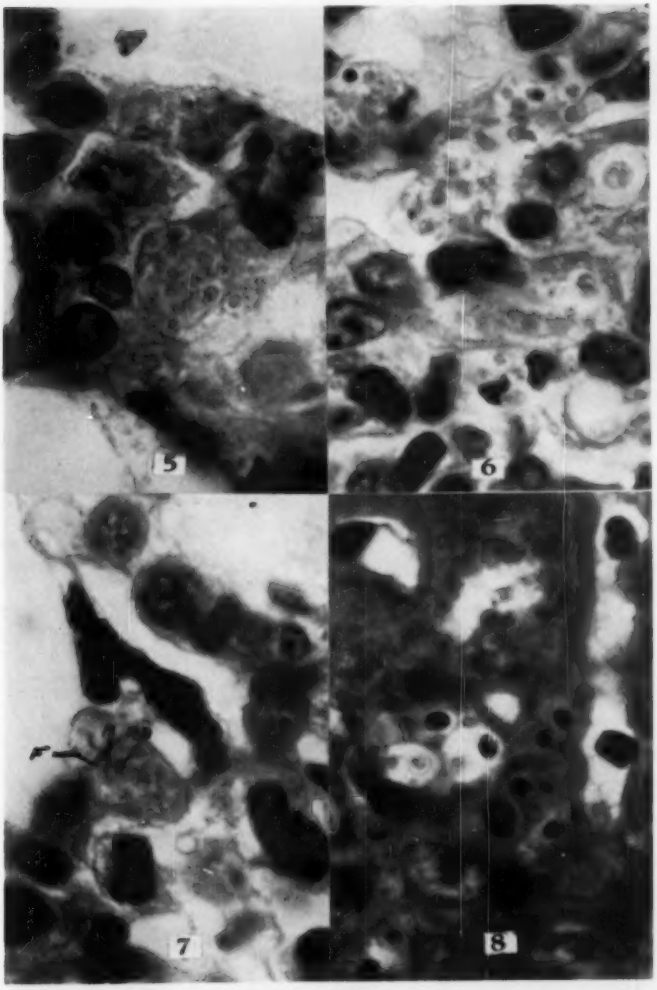
FIGS. 1–4 of the present paper are taken from the same preparation used to illustrate my 1942 paper. They show the appearance and structure of the nucleus with a densely stained nucleolus within the very delicate nuclear membrane. The many nuclei in the peripheral layer of cytoplasm in the young sporangium (FIGS. 1, 2), the multinucleate condition of the protospore (FIG. 3) and the uninucleate condition of the definitive spore (FIG. 4) are shown. The resting nuclei are 0.8–1.5 μ in diameter and the nucleolus is 0.3–0.4 μ.

Histoplasma

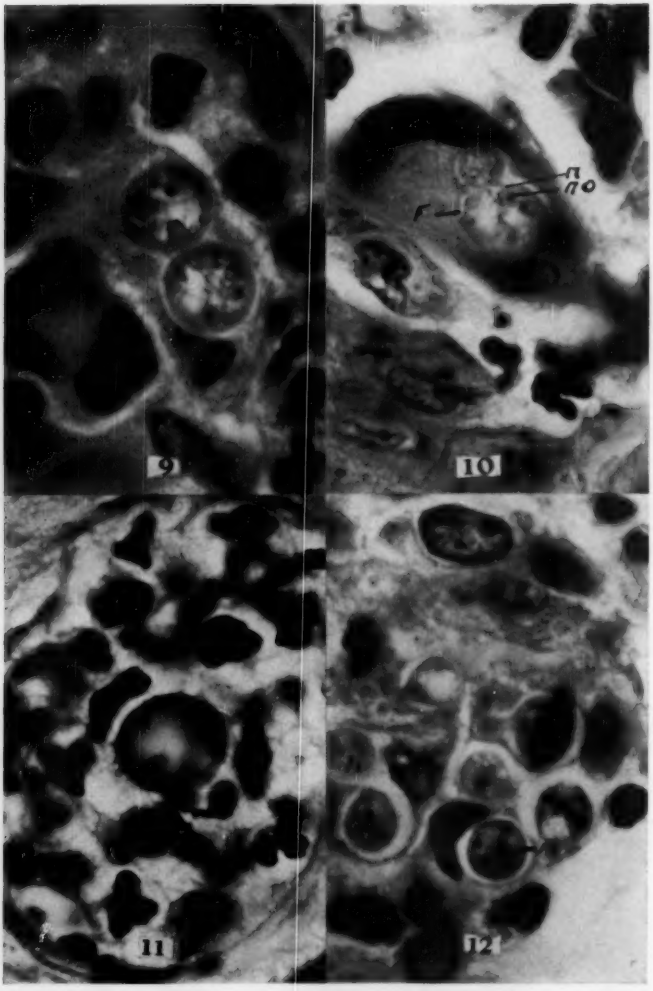
The small size of *Histoplasma capsulatum* and the correspondingly small size of the nucleus (0.5–0.8 μ) make the latter difficult to illustrate in a photomicrograph. However, as FIGS. 5–8 indicate, and as may be determined by careful examination at various optical levels of a properly stained section, the yeast cells of *Histoplasma* are uninucleate and the nucleus has the characteristic structure of the fungus nucleus. Even large cells of *Histoplasma* and those of *H. capsulatum* var. *duboisii* are uninucleate, except during reproduction. This uninucleate condition may be of practical importance in the differentiation of *Histoplasma* from *Blastomyces* in the rare cases where a double infection has been suspected or when the unusual size of either fungus in certain cases has posed a diagnostic problem.

Blastomyces dermatitidis

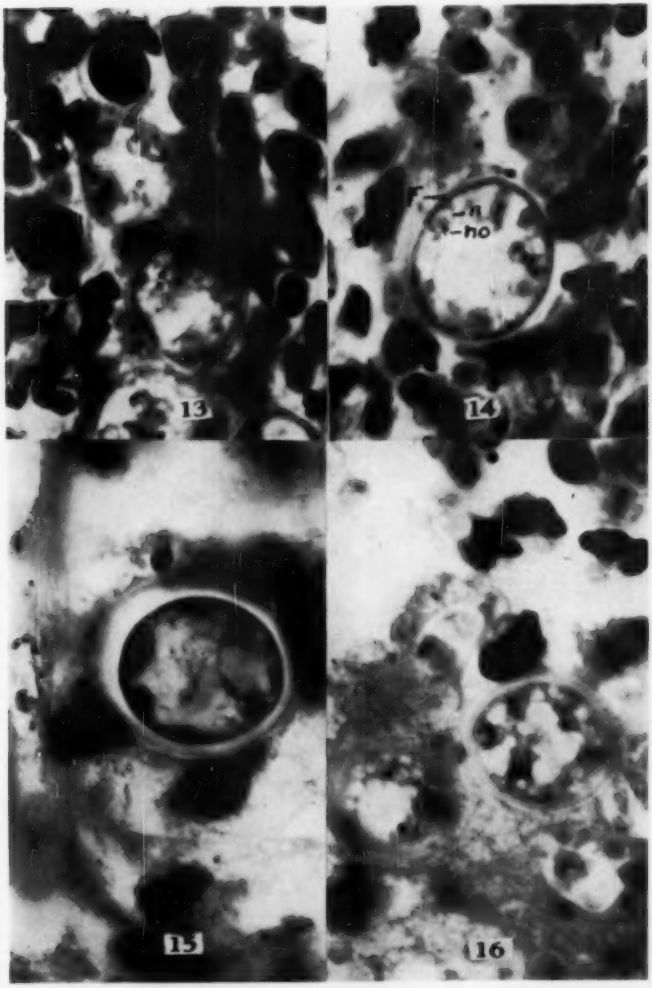
The cells of *Blastomyces dermatitidis* are always multinucleate. De Lamater (**5**, **6**) and Bakerspigel (**3**) have described and illustrated nuclear structures in *Blastomyces*. The nuclei are 1.0–1.5 μ in diameter, the nucleoli an estimated 0.2 μ. FIGS. 9, 10, 11 represent the fungus in a microabscess in dermal human blastomycosis from a piece of tissue obtained by biopsy which I processed and stained in 1931. The fungi in FIG. 12 are from mice experimentally injected with a mixture of *Blastomyces* and *Histoplasma*. In the experimental mixed infection,



FIGS. 5–8. *Histoplasma capsulatum.* Uninucleate cells. F—fungus cell wall, n—nucleus, no—nucleolus. × 1600.



FIGS. 9–12. *Blastomyces dermatitidis*. Multinucleate cells. F—fungus cell wall, n—nucleus, no—nucleolus. × 1600.



FIGS. 13–16. *Paracoccidioides brasiliensis*. F—fungus cell wall, n—nucleus, no—nucleolus. × 1600.

differentiation of the two fungi was easily made on measurements and morphologic evidence because each of the fungi occurred only in typical size and form.

Paracoccidioides brasiliensis

P. brasiliensis, as previously shown by Drouhet and Zapater (**7**), is multinucleate and the nuclei have the typical structure and appearance of fungus nuclei. They are 1.8–2.2 μ in diameter and are illustrated in FIGS. 13–16.

Cryptococcus neoformans

Dr. Ned Feder, using mice infected in this laboratory, has recently demonstrated the nuclei of *C. neoformans* by using the "freeze substitution" technic (**9**). Only a single nucleus is present, except, of course, during the process of reproduction. Sections prepared as described under Methods show considerable variation in size of the nucleus, depending upon the size and stage of development of the fungus cell (FIGS. 17–20).

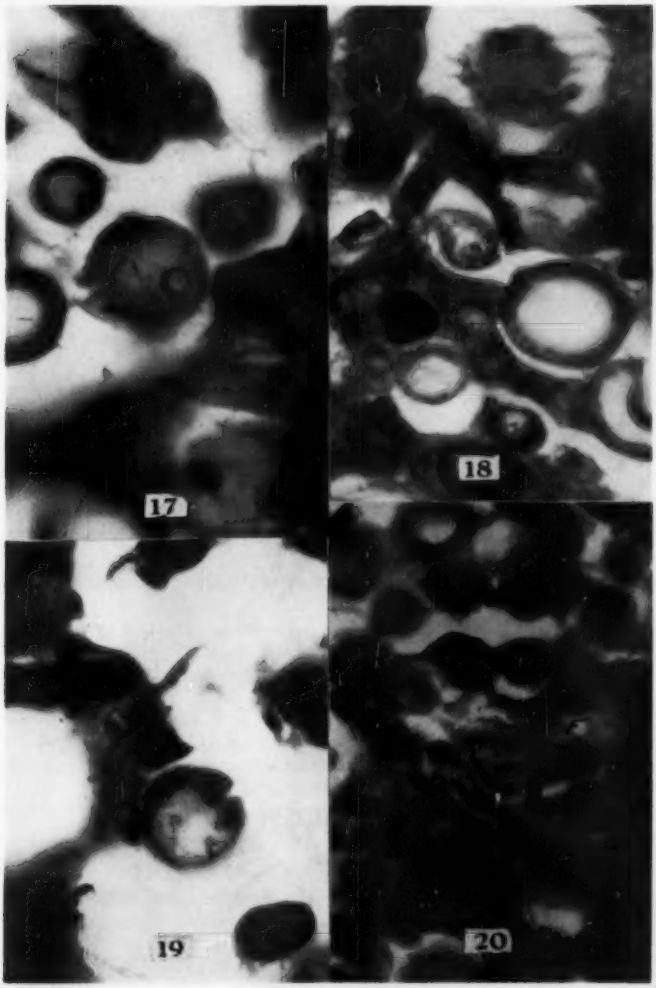
DISCUSSION

The nuclear condition of the fungi causing human disease is of more than academic interest. Under favorable conditions of fixation it may be possible to differentiate between *Histoplasma* and *Blastomyces,* for example, in a section showing fungus cells of varying or intermediate sizes in cases where identification of the atypical organism is in doubt because of lack of cultures. In such a favorable preparation, it should also be possible to answer the question which has been raised in a few instances of whether a mixed infection exists. The reliability of this method of differentiating *Histoplasma* and *Blastomyces* is dependent upon a minimum of the distortion and shrinkage which usually accompanies formalin fixation and rapid dehydration and infiltration in the routine histologic technics.

In some of the preparations studied, nuclei appeared to be in various stages of mitotic division, or reconstruction of the resting nucleus after division. The chromatic reticulum in the nucleus illustrated in FIG. 20 probably represents such a stage.

SUMMARY

The morphologic integrity of the fungus nucleus is lost in histologic preparations, unless all stages of fixation, dehydration, infiltration and



FIGS. 17–20. *Cryptococcus neoformans.* 17 and 18. Tangential sections. 19. Old cell with characteristic break in wall at point where budding continues. 20. Budding cell, one bud (b) cut in cross section. Note chromatic reticulum in nucleus. F—fungus cell wall, n—nucleus, no—nucleolus, b—bud. Figs. 17–19, × 1600. Fig. 20, × 1000.

staining are carefully conducted. Fixation with a modification of Bouin's fixative, dehydration and infiltration by slow and graded steps and staining with Heidenhain's iron-alum haematoxylin demonstrate small nuclei in the tissue phases of some pathogens of man with a minimum of apparent distortion and shrinkage.

Coccidioides, Blastomyces and *Paracoccidioides* are multinucleate. *Histoplasma* and *Cryptococcus* are uninucleate. The nuclei of these fungi are characteristic of those described and illustrated in many other fungi and consist of a delicate nuclear membrane, a vacuolated nucleoplasm and a densely stained single nucleus.

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ACTIVATION OF NEUROSPORA ASCOSPORES BY ORGANIC SOLVENTS AND FURANS[1, 2]

A. S. Sussman, R. J. Lowry and E. Tyrrell

It has been shown previously (Emerson, **1948**; Sussman, **1953**) that dormant ascospores of *Neurospora* respond to very low concentrations of heterocyclic 5-membered ring compounds, including certain thiophene and furfural derivatives, and pyrrole. Although over 100 other substances were tried, only compounds with the above structure were active, with the exception of ethyl ether which was weakly effective in amounts 100-fold greater than were required when heterocyclics were used.

Since that time, it was discovered that temperatures between 40 and 48° C, although insufficient to activate the ascospores, enhanced their sensitivity to chemical activators (Sussman, **1954a**). Therefore, it seemed reasonable to expect that a more detailed investigation of the chemical specificity involved in the activation process might be possible by the use of this technique. Moreover, the observation that ethyl ether was also an activator suggested the possibility that other organic solvents might also serve in this way. That this surmise was correct is shown by the following experiments, which expand the list of chemical activators to include aliphatic alcohols, esters, and ketones, in addition to the furans used previously and in the present studies.

MATERIALS AND METHODS

Strains 377.4 and 377.5 of *Neurospora tetrasperma* were used in this work and were crossed and grown at 25° for about a month before being harvested. The growth and harvest of ascospores were accomplished as described by Goddard (**1935**), except that stock cultures were transferred alternately between potato-dextrose agar and 0.5% yeast-extract medium containing 0.5% sucrose. After being harvested the

[1] These researches were greatly aided by a grant from the National Science Foundation and the Phoenix Project of the University of Michigan. Paper No. 1093 from the Department of Botany, University of Michigan, Ann Arbor, Michigan.

[2] The authors would like to thank Dr. Yurchenko of the Eaton Laboratories, Norwich, New York, for his cooperation in sending the furans used in these experiments. Also, the valuable assistance of Dr. Berta von Böventer-Heidenhain and Lewis Hahn is gratefully acknowledged.

ascospores were suspended and shaken in 0.8% ethylene diaminetetraacetic acid (EDTA) which had been brought to pH 7.1 with NaOH. By this means, conidia are killed and the ascospores dispersed without any effect upon their viability, even after prolonged exposure to this chelating agent (Sussman, **1954b**). This treatment serves also to increase the uniformity of the different spore lots, since contaminant trace metals on the cell surface are replaced by the sodium ions of the EDTA solutions. The EDTA was removed by washing the spores in four changes of distilled water before they were used. In all cases, unless otherwise mentioned, ascospores were suspended in EDTA at least two weeks before being used. In the experiments which follow, except when noted otherwise, ascospores were used two to six months after they were harvested.

In order to clarify subsequent discussion, the following terminology of the different physiological stages of ascospore development has been used:

dormant—those cells that have not been treated to induce them to germinate;

activated—those cells that have been treated so that under the appropriate conditions of incubation they will germinate;

germinated—those cells that have been activated and incubated until germ tubes have protruded.

Compounds [2] were tested for their ability to activate ascospores by incubating 1 ml of the chemical with an equal volume of ascospores such that their final concentration was 0.5 mg/ml dry weight. This weight corresponds to a reading of 90 with the Klett-Summerson colorimeter with a blue filter (Klett #42). Dormant ascospores as well as those that were "heat-sensitized" (Sussman, **1954a**) by exposure to a temperature of 46° for 30 minutes were used. Incubation was carried out on a reciprocal shaking machine at a rate of 100 oscillations per minute with a throw of 10 cm for at least 4 hours at 25°. After this time, the cells were killed by the addition of several drops of formalin and the amount of germination determined. At least 500 spores were counted and the average of duplicate counts was used in computing the percentage germination.

RESULTS

The results of experiments wherein derivatives of furan were tested for their ability to activate "heat-sensitized" ascospores are presented in TABLE I. The ten compounds listed were the only ones that were active, except for those that had been reported previously (Sussman,

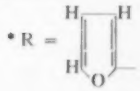
1953; Emerson, 1954). Of the compounds tested in the present experiments, furfuraldehyde diacetate and 2-furfuryl n-butyrate were the most active.

It had been noted in some experiments with organic compounds dissolved in ethyl alcohol that the solvent alone seemed to activate ascospores of *Neurospora*. Consequently, "heat-sensitized" and unsensitized spores were treated for two hours with several concentrations of methanol and ethanol in order to examine this possibility. After this time the

TABLE I

COMPOUNDS WHICH ACTIVATE NEUROSPORA ASCOSPORES. SPORES WERE HEAT-SENSITIZED FIRST BY EXPOSURE TO 46° C FOR 30 MINUTES. DASHES INDICATE CONCENTRATIONS THAT WERE NOT TRIED

Compound*		Heat-sensitized percentage germination				
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Ethyl-2-furfuryl ether	R. $CH_2OC_2H_5$	42	12.3	0	—	—
Methyl (2-furyl) acrylate	R. $CH{=}CHOOCH_3$	0	4.9	0	—	—
Methyl (2-furyl) propionate	R. $CH_2CH_2COOCH_3$	0	4.7	0	—	—
Methyl 2-furoate	R. $COOCH_3$	23.4	49	16.8	—	—
Furfuraldehyde diacetate	R. $CH(OCOCH_3)_2$	—	8.2	54.5	22.2	0
2-furaldehyde semicarbazone	R. $CH{=}NNHCONH_2$	60.4	30.4	0	—	—
2-N-propyl furan	R. $CH_2CH_2CH_3$	—	3.1	2.0	1.8	—
N-propyl furoate	R. $COOCH_2CH_2CH_3$	—	19.3	5.0	0	—
2-furfuryl-isobutyrate	R. $CH_2OCOCH(CH_3)_2$	—	0	25.4	3.6	—
2-furfuryl.N-butyrate	R. $CH_2OCOC_3H_7$	—	13.4	54.7	8.7	—



spores were washed by centifugation at about 3000 × g in three changes of distilled water and incubated in water at 25°. Germination was counted as described in the previous section and the results outlined in TABLE II. These results establish that methanol and ethanol are indeed excellent activators when high concentrations are used and that the latter compound is effective over a broader concentration range than is the former. Moreover, it seems clear that "heat-sensitization" induces a greater response to these solvents as well as to furans.

TABLE II

EFFECT OF METHANOL AND ETHANOL UPON ACTIVATION OF NEUROSPORA ASCOSPORES. "HEAT-SENSITIZED" OR UNTREATED DORMANT ASCOSPORES WERE EXPOSED TO THE SOLVENTS FOR 2 HOURS, AFTER WHICH THEY WERE WASHED AND RESUSPENDED IN WATER

Substance	Concentration	Percentage germination	
		Sensitized	Unsensitized
Methanol	1 M	2	0
	2 M	45	0
	5 M	55	0
	10 M	91	89
	15 M	0	0
Ethanol	1 M	63	2
	2 M	90	36
	5 M	83	42
	10 M	45	48
	15 M	0	0

The toxicity of high concentrations of alcohols to living organisms suggested that exposures of too long duration might kill the ascospores. Therefore, in order to define the optimal time for treatment with these substances, spore suspensions were exposed to methanol and ethanol for different periods of time and were washed and incubated as before. The results are given in TABLE III and indicate, as in the previous experiment, that ethanol is effective over a broader range of concentrations than is methanol. The optimum time of exposure varies inversely with the concentration used and prolonged exposure to high concentrations (10M and 15M) results in the killing of the ascospores.

Some of the higher alcohols were also used in several concentrations, and for various times, with the results shown in TABLE IV. Of these

TABLE III

TIME-COURSE OF ALCOHOL-ACTIVATION OF NEUROSPORA ASCOSPORES. "HEAT-SENSITIZED" ASCOSPORES WERE USED

Substance	Concentration	Percentage germination time in chemical (min.)						
		0	5	15	30	60	120	240
Methanol	1 M	0	2	0	5	3	2	—
	10 M	0	11	50	85	91	91	—
	15 M	0	28	14	0	0	0	—
Ethanol	1 M	0	8	11	20	32	63	80
	10 M	0	52	76	88	86	45	0
	15 M	0	64	82	76	43	0	—

compounds, i-propanol, n-propanol, and n-butanol were most effective although n-amyl alcohol showed considerable effect.

This survey of solvents was extended to include acetone which, as the results in TABLE V disclose, is an activator in concentrations of 1 to 5 M. In addition, benzyl alcohol and benzyl chloride at 5×10^{-4} M activated to the extent of 24 and 32%, respectively, after an exposure of 18 hours. In the latter case, benzene was used as a solvent, for it was found not to affect germination.

These experiments were extended to include other non-polar solvents including ketones and esters. When used in concentrations below

TABLE IV

EFFECT OF HIGHER ALCOHOLS UPON ASCOSPORE GERMINATION

Substance	Time	Concentration	Percentage germination	
			Sensitized	Unsensitized
i-propanol	½ hr	5 M	25	0
	3 hr	10 M	74	5
n-propanol	3 hr	1 M	15	21
	1 hr	2 M	10	0
	½ hr	5 M	42	0
	¼ hr	10 M	81	20
n-butanol	½ hr	5 M	29	8
	½ hr	10 M	47	14
s-butanol	½ hr	5 M	2	0
	1 hr	10 M	2	0
t-butanol	1 hr	5 M	4	0
	1 hr	10 M	3	0
n-amyl alcohol	½ hr	0.15 M	7	4
	½ hr	0.30 M	24	3

1×10^{-2} M these substances showed very little effect, so that the experiments to be reported were carried out with the anhydrous solvents. Ascospores suspended in water were heat-sensitized, after which the water was removed by filtration through a sintered glass filter (fine porosity). The spores were then resuspended in the solvent to be tested, for varying amounts of time, washed five times in water, and incubated for three hours as described before. Controls treated with furfural, or with water, were run with every experiment, thereby providing a check upon the viability of the spores and upon the amount of activation induced by the heat-sensitization. As TABLE VI reveals, sev-

TABLE V

EFFECT OF ACETONE UPON ACTIVATION OF NEUROSPORA ASCOSPORES

Concentration of acetone	Percentage germination time in acetone (minutes)					
	0	30	60	90	120	180
Sensitized spores:						
1 M	0	11	12	16	15	0
2 M	0	13	14	38	53	76
5 M	0	3	0	0	0	0
10 M	0	0	0	0	0	0
Non-sensitized spores:						
1 M	0	2	0	0	0	0
2 M	0	4	2	10	15	30
5 M	0	10	2	0	0	0
10 M	0	0	0	0	0	0

TABLE VI

EFFECT OF KETONES AND ESTERS UPON ASCOSPORES OF NEUROSPORA. HEAT-SENSITIZATION WAS ACCOMPLISHED AT 46° C FOR 30 MINUTES. THE TIMES RECORDED BELOW WERE FOUND TO BE OPTIMAL WHEN ANHYDROUS SOLVENTS WERE USED

Compound	Time of exposure	Percentage germination
Ethyl acetate	15 minutes	22.1
Ethyl butyrate	30 minutes	37.2
3-pentanone	5 minutes	10.9
4-heptanone	30 seconds	12.5
4-methyl,2-pentanone	30 seconds	12.0
2-octanone	1–2 minutes	1.4
5-nonanone	2 minutes	9.2

TABLE VII

INFLUENCE OF DATE OF HARVEST UPON ACTIVABILITY OF NEUROSPORA ASCOSPORES BY FURFURAL. EXPERIMENTS PERFORMED IN APRIL, 1956

Age (months)	Concentration of furfural	Percentage germination	
		Sensitized	Unsensitized
11	1×10^{-3} M	83	71
	1×10^{-4} M	86	65
8	1×10^{-3} M	81	73
	1×10^{-4} M	73	69
4	1×10^{-3} M	56	50
	1×10^{-4} M	26	21
2	1×10^{-3} M	59	28
	1×10^{-4} M	79	29

eral of the ketones, as well as ethyl acetate and ethyl butyrate, activate ascospores of *Neurospora*. Although the ketones tried, with the exception of 2-octanone, were also effective, they were less so than the esters. On the other hand, the use of the anhydrous solvents presented difficulties in that the spores were unevenly distributed in suspension. Therefore, it is possible that more uniform exposure to these materials would have greater effect. As in the case of the alcohols tested, the ketones and esters are toxic upon prolonged incubation with the spores.

Effect of age. Emerson (**1948, 1954**) had noted that the age of the ascospores influenced their response to furfural. Therefore, experiments were performed wherein ascospores of defined age were treated with furfural after being "heat-sensitized." As can be seen in TABLE VII, the older ascospores were activated more readily before being sensitized than were the more recently harvested ones.

TABLE VIII

EFFECT OF AGE UPON THE RESPONSE OF ASCOSPORES OF N. TETRASPERMA TO ACTIVATION BY ETHANOL. FINAL CONCENTRATION OF ETHANOL WAS 5 M AND ASCOSPORES WERE TREATED WITH EDTA FOR 1 MONTH BEFORE USE. SENSITIZED AND NON-SENSITIZED CONTROLS SHOWED NO GERMINATION

Time in ethanol (hours)	Non-sensitized[a]		Sensitized[b]	
	Age (months)	Percentage germination	Age (months)	Percentage germination
1	11	31	13	72
2		64		80
1	8	15	10	18
2		49		22
1	4	10	6	32
2		37		50
1	2	4	4	9
2		15		11

[a] Experiments performed April 3 and 4, 1956.
[b] Experiments performed May 29, 1956.

That the sensitivity of spores to activation by solvents changes with age was shown by experiments wherein ascospores of different age were exposed to 5 M ethanol for varying periods of time and incubated as usual. All these spores had been shaken in 0.8% versene (pH 7.1) for 1 month preceding this experiment, after which they were washed as before and resuspended in water. The data in TABLE VIII attest to the

fact that age is an important factor when both sensitized and unsensitized ascospores are used.

Ordinarily, heat-sensitization at temperatures between 40° and 48° does not result in the activation of ascospores, unless furfural is added (Sussman, **1954a**). However, it was observed during the course of these experiments that some lots of spores showed a high percentage of germination, even in the absence of chemical activators. In order to assess the influence of age upon activation by high temperatures, several lots of spores were maintained at 46° for 30 minutes. Thereupon, the spores were incubated as before, and the percentage germination determined. The results, presented in TABLE IX, disclose that recently harvested ascospores are not activated by temperatures as low as 46° whereas those that are almost four years old germinate to the extent of 24%. Asco-

TABLE IX

EFFECT OF AGE UPON THE ACTIVATION OF ASCOSPORES AT 46° C. EXPERIMENTS WERE PERFORMED IN APRIL AND MAY OF 1958

Age (months)	Percentage germination
46	24.0
26	2.4
21	0.5
19	6.7
7	4.8
6	0
3	0

spores of an age intermediate between these are only slightly activated at this temperature.

DISCUSSION

Until the present work, a considerable degree of specificity in the structure of activators was suggested. Thus, furfural and furfuryl alcohol (Emerson, **1948**), as well as several other furans, pyrroles and thiophenes (TABLE I; Sussman, **1953**; Emerson, **1954**), were reported to be effective in very low concentrations. These data suggested that the following conditions had to be met before activity was assured:

1. a heterocyclic nucleus containing one oxygen, nitrogen, or sulfur atom in the ring, and
2. a sidechain in the 2-position containing a non-polar terminal portion.

However, an exception to these rules had been noted by Sussman (**1953**) in that ethyl ether activated ascospores of *Neurospora*, and subsequently

Emerson (**1954**) showed that benzaldehyde also acted in this way. The former observation led to the experiments reported here which show that several solvents, in relatively high concentration, activate ascospores of *Neurospora*. Among the substances in this category are aliphatic alcohols, ketones, and esters which, in too high concentration, may be toxic. In addition, Emerson (**1954**) has stressed the importance of aldehydes as activators so that non-polar groups, in general, appear to be most effective. That the mechanism by which these substances break the dormancy of ascospores is probably different from that by which heterocyclic compounds operate is suggested by the disparity in concentrations required. Thus, 1×10^{-3} M furfural is routinely used for activation, but more than one thousand times as much of the alcohols and other solvents must be used.

That the stimulatory effect of solvents upon germination is not restricted to *Neurospora* has been shown in several cases. For example, ethanol, methanol, and ether vapors stimulated the germination of conidia of *Aspergillus flavus* (Duggar, **1901**), and Brown (**1922**) reported that esters like ethyl acetate, ethyl citrate, or ethyl malate aided the germination of *Botrytis cinerea*. It should be noted that the effect of the solvents used in the above cases is that of increasing the amount of germination, in that the conidia of these species do not appear to have a period of dormancy. On the other hand, Noble (**1924**) showed similar effects upon spores of *Urocystis tritici* using acetone, benzaldehyde, or salicylaldehyde.

In nature, the most likely means by which dormant ascospores of *Neurospora* may be induced to develop is through exposure to furfural and other furans. The small quantities of these substances that are required, and their wide distribution, make this most probable. In fact, Dunlop and Peters (**1953**) point out that furfural is one of the most ubiquitous of organic compounds, inasmuch as it is a breakdown product of pentosans and similar plant products. Therefore, most terrestrial habitats would furnish the means by which these ascospores can be activated. The recent work of Tylutki (**1958**) suggesting that furfural may activate ascospores of *Gelasinospora* fortifies the notion that others of the coprophilous ascomycetes may respond to this and related compounds. In addition, the finding that nonanal, or a related compound, may be an endogenous activator of uredospores of wheat rust (French and Weintraub, **1957**), taken together with the cases cited above, suggests that effects similar to those reported for *Neurospora* may be of general significance in the fungi.

Although the mechanism of action of chemical activators is not known, they appear to be of two different classes. First, there are the

furans, for which some degree of structural specificity obtains. Furthermore, these compounds are effective in amounts which range between 1×10^{-4} M and 1×10^{-2} M. Then, there are the aliphatic alcohols, ketones, and esters, which are required in concentrations greater than 1 M and which appear to have no structural similarity beyond their non-polar-active group. Heat-sensitization of ascospores is usually necessary for the optimal activity of both groups of compounds to be demonstrated.

Recent work has suggested some models for the mechanism of action of these substances. For example, solvents like ethyl ether, several ketones, and esters have been shown to produce a large stimulation of plastid phosphatidase-C activity (Kates, **1957**). The mechanism proposed by this author is that the surfaces of the plastid and substrate have been made lipophilic by such treatment, thereby making them more mutually attractive in a water medium. However, a modification of this model has to be introduced when *Neurospora* is considered because, according to this mechanism, alcohols should not be effective. Augmented catalase activity in yeast (the "Euler effect") was obtained by Kaplan (**1954**) after treatment with aliphatic alcohols, ketones, and aldehydes. Of particular interest was the observation (Kaplan, **1955**) that surface-unfolded and intracellular catalases are activated by short treatment at 50–56°. The conclusion of this author was that these chemicals caused enzyme alteration by being absorbed at an intracellular interface, thereby modifying the properties of this enzyme, perhaps by its being desorbed into solution. However, the application of models such as these must await the discovery of the key reaction(s) triggering activation in ascospores of *Neurospora*.

SUMMARY

Ten furans have been shown to activate ascospores of *Neurospora tetrasperma*. Of the compounds tested in these experiments, furfuraldehyde diacetate and 2-furfuryl n-butyrate were most active. In addition, aliphatic alcohols, ketones, and esters were effective when amounts greater than 1 M were used. These compounds were toxic when used in too high concentrations, or when kept in contact with ascospores for protracted periods of time. Age was shown to influence the response to chemicals in that freshly harvested spores germinated only poorly in their presence, except when pre-treated at 46°. By contrast, a larger percentage of older spores germinated in the presence of chemicals without such treatment. Moreover, spores that were four years old could

be activated at lower temperatures than those required by more recently harvested ones. These results suggest that there are at least two classes of chemical activators, including heterocyclics active in very low concentrations and aliphatic non-polar compounds which are effective in concentrations greater than 1 M.

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STUDIES ON THE ISOLATION AND GROWTH OF PLANT RUSTS IN HOST TISSUE CULTURES AND UPON SYNTHETIC MEDIA.[1] I. GYMNOSPORANGIUM

VICTOR M. CUTTER, JR.

(WITH 4 FIGURES)

The obligate parasitism of the plant rusts has been long known and little understood. It has been recognized since the time of DeBary that the vegetative stages of these pathogens would not continue independent growth apart from their respective host cells. Before 1930 the gamut of enrichment media, host-cell extracts and supplementary growth factors had been employed in futile attempts to discover the precise factors responsible for the obligatory parasitism of the Uredinales. With two possible exceptions the reports concerning attempted isolation in pure culture indicate clearly that the usual methods employed for the isolation and culture of facultative parasites are inadequate to maintain these obligates.

A lengthy historical review of the various attempts to grow rusts upon artificial media would serve little purpose here, since, as far as can be determined, all such experiments up to 1951 have been fruitless. The report by Ray (36) in 1901 of the growth of a number of species

[1] The portions of the work reported here which were carried out from 1949 to 1952 were aided by a Rockefeller Foundation grant to the Department of Plant Science of Yale University. The investigation subsequent to 1952 has been supported by a grant from the National Science Foundation (NSF-G219).

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of rusts on sterilized carrots and upon various host-cell decoctions has never been substantiated and, in the light of our present knowledge, hardly seems credible, while the casual mention by Gretschushnikoff (**16**) in 1936 of the saprophytic growth of four species of *Puccinia* for 12 days is open to questions of interpretation and apparently has not been subsequently confirmed. Arthur (**2**) has presented a detailed summary of this work to 1929 and from that time until the appearance in 1951 of the account by Hotson and Cutter (**22**), of the isolation in axenic culture of *Gymnosporangium juniperi-virginianae*, no reports of the continued culture of a plant rust in the absence of its host plant were published. The excellent critical reviews by Allen (**1**) and Yarwood (**45**) summarize the recent work on this problem so concisely that reiteration would be superfluous.

In 1949 Dr. Georges Morel suggested to the author that the cedar-apple-rust gall on *Juniperus* should provide ideal experimental material for adaptation studies on systemic rust mycelium growing in a host-callus culture. This suggestion was based on his earlier unsuccessful attempts to inoculate callus cultures of *Crataegus monogyna* Jacq. with *G. clavariaeforme*. As he pointed out (**31**), a plant organ systemically infected by the rust under natural conditions would be easily handled aseptically and should produce callus in which the rust might develop. This hypothesis was directly responsible for our first successful culture of callus tissue infected by a plant rust and, shortly thereafter, for the first report of the culture of a plant rust apart from the tissues of its host (**22**). Here we indicated that, after the fungus had been isolated, the mycelium was tested by inoculating uninfected callus cultures of *Crataegus coccinea*, upon which, subsequently, typical intercellular mycelium and roestelia of *Gymnosporangium* developed. Later Cutter (**9**) reported that 3 additional strains of *G. juniperi-virginianae* had been isolated in axenic culture by the same technique and had been maintained by repeated subculture for 6 months without loss of their ability to reinfect the alternate host. At that time certain speculations concerning the essential nutrient requirements of the rusts were also advanced.

In his doctoral dissertation, dated December, 1951, which was subsequently distributed on microfilm (**20**) and in a later publication (**21**), Hotson discussed some of his observations on the induction of callus tissue from the galls of *G. juniperi-virginianae* as well as the development of teliospores and of intercellular rust mycelium in these cultures.

Shortly after our first report appeared, Dr. Joseph C. Gilman and Dr. Frank D. Kern independently pointed out, in personal communica-

tions to the author, that there must be some question about the identification of the isolated fungus since *Gymnosporangium juniperi-virginianae* was not known to parasitize the genus *Crataegus* as an alternate host. Both these correspondents suggested that in all probability the organism was actually *Gymnosporangium globosum* Farl., which commonly attacks *Crataegus,* and which, of course, occurs in the same habitats and frequently on the same coniferous host plant with *G. juniperi-virginianae.* Since at that time no callus cultures of *Pyrus* were available for testing we had not been able to test the infectivity of this isolate on its usual host. As will be pointed out below, subsequent inoculation tests upon a number of strains of *Crataegus* and *Pyrus,* under both field and greenhouse conditions, have not completely clarified the question of the specific identity of this isolate, since it consistently parasitizes both rosaceous hosts.

In addition to direct attempts to culture these organisms apart from their hosts, numerous published studies on the factors affecting germination and dormancy of the various rust spores provide additional evidence that their failure to grow upon artificial media resides in metabolic peculiarities inherent in their vegetative growth systems rather than in the biochemical systems controlling spore dormancy and germination. The recent success of virologists in growing many viruses in plant and animal tissue culture and the work of Morel (**31**) on the growth of obligate plant parasites in detached tissue cultures of their host plants have indicated the possibilities of an indirect approach to axenic rust culture.

Morel's earlier attempts, and our own, to establish tissue cultures by the classic techniques and then to inoculate them with rust spores, in the hope of establishing an infection, indicated that this approach would not be practical for large-scale experiments. The difficulty of obtaining sufficient quantities of sterile rust spores to initiate such infection and the negligible rate of infection of tissue cultures in the preliminary trials, plus the high incidence of contamination with saprophytic fungi which are almost invariably present in the sporulating pustules of field-collected rusts, proved very discouraging. Attempts to surface-sterilize mature rust spores resulted in almost complete loss of viability, and the use of immature spores from unopened pustules immediately limited the amount of inoculum available. The work of Heim and Gries (**17**) offered further evidence of the impracticability of this approach. Sporadic attempts have been made subsequently to modify this technique because it offers the best hope of culturing those rust fungi which do not form systemic infections, but no positive results have been obtained.

In contrast to this, the use of field-collected plants, systemically infected under natural conditions, as the source of tissue cultures has been most profitable and all the work reported here is based on this technique.

The use in this investigation of the term "systemic infection" is perhaps somewhat broader than that currently favored by uredinologists. This term is employed here in reference to a rust infection in which an entire organ or a significant portion of the plant is invaded by the rust mycelium, or where the mycelium perenniates for more than one full growing season in a portion of the host. It does not necessarily imply that the entire plant is invaded by mycelium although this may be true. A "tissue culture" is defined as a mass of cells, derived from any part of a plant, growing aseptically *in vitro* under conditions where subsequent growth is relatively undifferentiated. This is synonymous with White's concept (**43**) of a callus culture. An infected tissue culture is one with systemic rust mycelium parasitizing the host cells. Usually, when the tissue culture is established, no further differentiation of organs occurs and the callus mass proliferates indefinitely as an unorganized group of cells, portions of which may be transferred or subdivided at will. The term "axenic culture," employed by Yarwood (**45**), has been adopted when referring to cultures of rusts isolated from the host and growing in the mycelial condition on synthetic media, in the complete absence of other organisms, since the epithet "pure culture" as used by uredinologists frequently connotes a single species or physiologic race of rust growing upon its host.

When a dual-membered tissue culture was established efforts were directed towards inducing the rust to proliferate and grow as vigorously as possible on the assumption that any mutation or inherent variation in the direction of saprophytism might then operate selectively to enable the rust to become independent of its host. In addition, there was always the possibility that an adaptive enzyme system might be present which would allow saprophytic existence if it were properly encouraged or "trained." It seemed wise to maintain these dual-membered cultures for the longest possible period under diverse conditions in the hope of providing, by chance, the proper selective environment. This random approach was necessary for the simple reason that, in spite of the exhaustive attempts of the last fifty years to elucidate the essential factors controlling obligate parasitism within this group, there were practically no clues to the precise nutritional ingredients which any rust obtained from its living host. In other words, the key substance or metabolite, if indeed such exists, was completely unknown. This procedure could

be justified on the basis that until a rust had been induced to grow apart from its specific host the metabolic block or requirement that resulted in obligatory parasitism could not be detected.

MATERIALS AND METHODS

TABLE I enumerates the rust fungi and their host species which were studied during this investigation. Only those experiments where callus tissue was successfully induced on the infected hosts will be discussed. The synonymy followed conforms to that used in the first edition of Arthur's Manual (**2**) for the rust species and to the 8th edition of Gray's Manual of Botany (**12**) for the hosts.

Rusted material was collected in the field and brought to the laboratory as quickly as possible. If more than a few hours' delay was anticipated between the collection of the rust and the preparation of the cultures the material was refrigerated in a portable icebox. In some instances woody materials were held in this way as long as 6 days at 10° C.

The telial galls were prepared for culture by detaching them from the twigs and immersing the entire gall in the sterilizing agent for 5–20 minutes. Diluted and undiluted Clorox, 3% hydrogen peroxide, 2% bichloride of mercury, 1% Lysol, 70 and 95% ethyl alcohol, 0.5% Captan, 0.75% Fermate and 1% Na benzene dodecyl sulfonate were used for surface sterilization during the early experiments. In all cultures started after 1951 full-strength Clorox was used, since it controlled the superficial contaminants well, and at the same time did not markedly inhibit the ability of the underlying tissues to regenerate callus. No callus tissue ever formed following treatment with bichloride of mercury, Lysol, Captan or Fermate; and Na benzene dodecyl sulfonate, while very effective in controlling bacterial contamination, was virtually without effect in suppressing growth of various imperfect fungi. Following immersion in the sterilizing agent the galls were rinsed in sterile distilled water and sliced aseptically, and cubes approximately 5 mm square were dipped in 1% ascorbic acid and planted in tubes containing 10 cc of the selected medium (FIG. 1, A). In the first experiments the outer suberized layer of the galls was stripped off before planting, but it was soon noted that much more vigorous callus formation occurred in the outermost layers of the gall cortex and consequently in most cultures started after 1950 the suberized epidermal layer was left attached to the cubes. The cubes were oriented in various positions relative to the agar surface, but no effect of polarity on the development of callus was detected.

TABLE I

CALLUS FORMATION AND CULTURAL HISTORY OF THE TWO-MEMBERED RUST-HOST TISSUE CULTURES INITIATED IN THIS STUDY

Rust species	Host species	Rust stage	No. of localities	Plant organ used	No. of cultures	No. forming callus	Prim. subcultures	Sec. subcultures	Rust isolated
Gymnosporangium clavipes (Cke. et Pk.) Cke. et Pk.	*Amelanchier canadensis* (L.) Medic.	aecia	1	fruits	47	0	—	—	—
	Pyrus arbutifolia (L.) L.f.	aecia	2	fruits	68	0	—	—	—
	Crataegus Phaenopyrum (L. f.) Medic.	aecia	1	fruits	26	0	—	—	—
	Juniperus virginiana L.	telia	2	stem galls	49	2	0	—	—
Gymnosporangium clavariaeforme (Jacq.) DC.	*Juniperus communis* L. var. *depressa* Pursh	telia	1	stems	8	0	—	—	—
Gymnosporangium globosum Farl.	*Juniperus virginiana* L.	telia	1	telial galls	63	2	1	0	—
	Crataegus crus-galli L.	aecia	1	leaf disks	20	0	—	—	—
	Crataegus Engelmanni Sargent	aecia	4	fruits	78	2	0	—	—
			2	stem galls	25	4	9	3	0
			3	leaf disks	97	0	—	—	—
	Crataegus Youngii Sarg.	aecia	2	leaf disks	43	0	—	—	—
Gymnosporangium juniperi-virginianae Schw.	*Pyrus coronaria* L.	aecia	3	stems	54	5	12	29	0
				leaf disks	90	0	—	—	—
	Pyrus malus L. (cultivated)	aecia	14	leaf disks	222	0	—	—	—
		pycnia	2	fruit	31	4	0	—	—
		aecia	2	stems	14	6	19	47	0
	Juniperus virginiana L.	telia	54	telial galls	13,504	358	530	1729	7

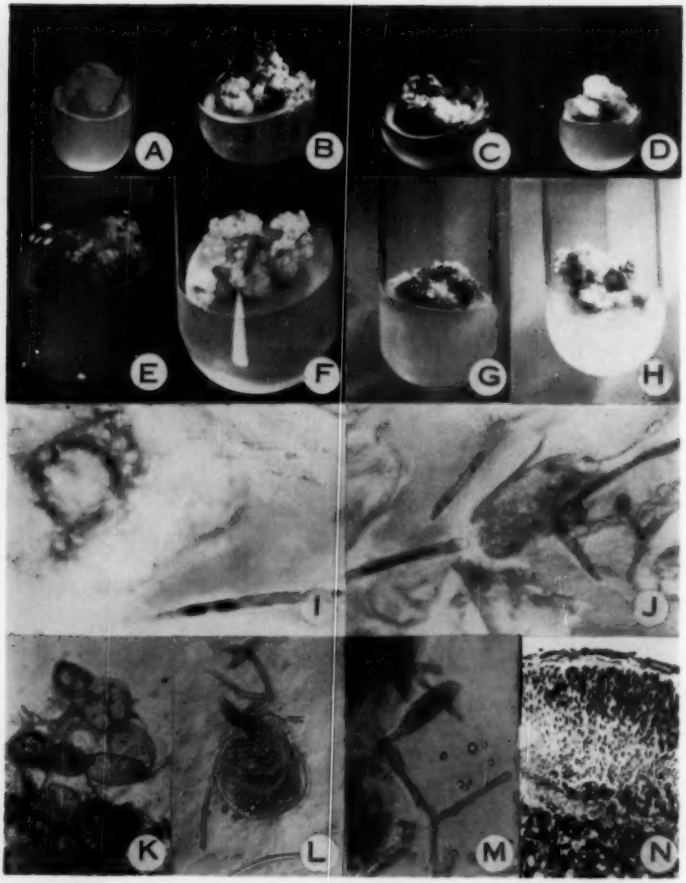

FIG. 1. A. Gall slice on Gautheret's agar prior to callus formation. × 1. B. Development of callus on gall slice, 75 days. × 1.5. C. Subculture of callus from B above, 90 days. × 1.5. D. Uninfected cambial callus of *Juniperus virginiana* on Gautheret's, 90 days. × 1. E. Callus systemically infected with *Gymnosporangium* after continuous subculture for 4 years on Gautheret's. × 2. F. Infected callus "habituated" on Gautheret's without IAA. × 2. G. Callus with aerial mycelium of *Gymnosporangium* starting to develop. × 1.5. H. Mycelium of *Gymnosporangium* overgrowing callus and invading medium. × 1.5. I. Binucleate mycelium and host-cell nucleus from *Juniperus* secondary callus subculture. DeLamater's basic fuchsin. × 830. J. Binucleate mycelium and haustoria from

Woody stem tissue of *Juniperus* and *Crataegus* systemically infected with *G. clavipes* and *G. clavariaeforme* was prepared by a modification of the method described by White (**43**) for cambial explants. The branches containing the galls or the entire infected woody stems were cut and the rough outer bark scraped off. The underlying cortex was then swabbed with 70% alcohol and briefly flamed. The stems and galls were then sawed transversely into quarter inch disks, with a sterile saw blade. These disks were laid on a flat surface and subdivided with a sterile chisel into small blocks approximately 3 mm square. Blocks selected from the cambial region were oriented in various planes on nutrient agar in tubes and pressed down to insure intimate contact. When uncontaminated these blocks showed callus formation within three weeks of planting. Gautheret's cambial technique (**13**) was utilized in the preparation of uninfected cambial callus from *Crataegus, Pyrus* and *Juniperus* used for testing the rusts isolated from these hosts.

Infected unripened fruits of *Amelanchier, Pyrus* and *Crataegus* were soaked 3 minutes in undiluted Clorox, washed in sterile water, sectioned into cubes and imbedded below the surface of Gautheret's agar. Infected callus was obtained only from the fruits of *Crataegus Engelmanni* and *Pyrus Malus*.

Many attempts were made to establish callus cultures from foliicolous infections but as indicated in TABLE I these were all unsuccessful. Disks of leaf tissue containing the rust pustules were cut with a 1 cm leaf punch and immersed in half-strength Clorox until the cut edges blanched. The disks were then rinsed quickly in sterile water and floated on the surface of liquid Gautheret's solution in test tubes, or were partially submerged below the surface of Gautheret's agar. The tubes were sealed and treated in the same manner as the callus cultures. In many cases the rust pustules continued to produce spores and frequently proliferated but there was no indication of callus formation on these leaf disks and rust mycelia never grew out from the cut surfaces.

The nutrient solution used in the routine initiation of callus tissue

Juniperus secondary callus subculture. Dark phase contrast. × 830. K. Triturated secondary callus subculture of *Juniperus* inoculated with NC-219-54. Note haustorium in central upper cell. Bright field, unstained. × 310. L. Triturated secondary callus subculture of *Juniperus* showing immature teliospore attached to intercellular mycelium on surface of callus cell. Dark phase contrast. × 310. M. Isolated teliospore and aerial mycelium produced on *Juniperus* callus after excision of telial horns. Bright field, unstained. × 310. N. X-section through primary callus subculture of *Juniperus* showing immature telium. Safranin and aniline blue. × 90.

on infected hosts was a modified Gautheret's solution made up from stocks as follows:

Green-coconut milk	150 cc
Trace element (Burkholder and Nickell's) sol.	1 cc
Complete vitamin sol.	10 cc
Sucrose	30 gm
Agar (Difco Noble)	10 gm
Indoleacetic acid 0.01% sol.	10 cc
Half-strength Knop's sol. to 1 liter	

This medium was mixed and autoclaved at 15 lbs pressure for 20 minutes. The approximate pH after autoclaving was 5.3 and it was not further adjusted except as noted below. The composition of the various solutions used in this formula are:

Burkholder and Nickell's trace element Sol. (**8**) *mg/l*

H_3BO_3	570
$MnCl_2 \cdot 4H_2O$	360
$ZnCl_2$	625
$CuCl_2 \cdot 2H_2O$	268
$Na_2MoO_4 \cdot 2H_2O$	252
$Fe_2(C_4H_4O_6)_2$	1825

Half-strength Knop's Sol. mg/l

$Ca(NO_3)_2 \cdot 4H_2O$	720
KNO_3	125
$MgSO_4$	125
KH_2PO_4	125
$FeCl_3$	1

Vitamin Sol. in μg/l

Thiamin	100
Riboflavin	50
Pyridoxine	50
Calcium pantothenate	200
Para-amino-benzoic acid	50
Nicotinamide	200
Choline	200
Inositol	200
Yeast nucleic acid	500
Folic acid	5
Biotin	1

This basic medium, which will be referred to as Gautheret's solution, was supplemented in various experiments with a wide variety of other substances designed to stimulate the growth of particular cultures, as noted below. A number of other media including those of White (**43**), Gautheret (**13**), Hildebrandt, Riker and Duggar (**19**), and Burkholder and Nickell (**8**) were employed to induce callus formation, but none of them yielded significantly better results.

Coconut milk was obtained from young green nuts shipped from Florida and was either used fresh or drained from the nuts upon receipt and frozen until use. The various stock solutions were stored at 4° under toluene until used. Indoleacetic acid (IAA) was filter-sterilized and added at a concentration of 10 μg/l to the autoclaved media.

All culture vessels were of Pyrex glass and were boiled in Alconox solution, rinsed repeatedly in distilled water and dried before use. The test tubes and flasks which contained the cultures were plugged with

nonabsorbent cotton and the excess cotton flamed off, after which the tubes were sealed with Parafilm. This proved efficient in preventing desiccation of the media during the long periods required for callus induction.

Because the formation of callus tissue is frequently encouraged by disturbing the normal orientation of tissues, stem segments were generally inverted and thrust partially below the surface of the solid culture medium. In all cases, however, some of the material was oriented in other directions relative to the axis of growth. Unless otherwise noted, the cultures were maintained at 20° with relative humidity of 56 per cent under constant illumination at 350 or 890 foot candles from "Warm White" fluorescent tubes. When callus tissue formed on a dual-membered culture in sufficient amount the tube was opened, the entire culture removed to a sterile petri plate and portions of the callus excised and returned aseptically to fresh media. Whenever subcultures were made in this way a portion of the callus was sectioned or triturated to determine whether rust mycelium was present in the new growth, and periodically thereafter the primary and secondary subcultures were examined for the presence of rust mycelium. The callus samples were cut fresh on a hand microtome in pith and stained in Cotton Blue or Trypan Blue according to the method described by Boedjin (**7**), or were fixed in F.A.A. and in Craf V (Sass, **38**) and embedded in Tissue Mat through tertiary butyl alcohol. Prior to fixation the calluses were separated into small masses approximately 3 mm in diam. Serial sections were cut at 7–20 μ and were stained with Safranin and Fast Green or Anilin Blue according to the schedule suggested by Popham et al. (**35**).

In the later experiments it was found more practical to place a bit of the callus in a small amount of Gautheret's solution in a Waring Blendor and triturate it until the cells were completely reduced. The homogenate was then centrifuged and the residue examined under the phase-contrast microscope for the presence of rust mycelium. This saved a great deal of time and aided immeasurably in the identification of the rust mycelium, which is frequently difficult to locate when present in only small amounts in sections.

Cytological preparations of the saprophytic rust mycelium were stained by the second Basic Fuchsin technique suggested by DeLamater (**10**), and by a modified Giemsa technique which involved fixation in acetone-free absolute methyl alcohol, after which the material was transferred directly to 0.25% aqueous Azur II for 2 min, destained in acid water and counterstained in 0.1% Eosin Y until a pink color developed.

The material was then dehydrated through alcohols, cleared in xylene and mounted in neutral Canada balsam. In both cases the fungus was prepared for observation by laying sterile squares of wettable cellophane upon the surface of nutrient-agar plates and placing the inoculum directly on the cellophane. After the colony had grown to the desired dimensions the cellophane squares, with fungus attached, were lifted from the nutrient substratum and carried through all subsequent procedures exactly as though the fungus were attached to a glass slide.

Since the primary purpose of this investigation was to prolong the culture of these rust-infected calluses and not to study their physiological behavior they were transferred only when the cultures showed signs of desiccation, or the growth rate of the callus declined. When a transfer was necessary the tube was opened, the mouth flamed and the entire callus lifted with flamed forceps to a sterile petri plate. It was then subdivided into portions about 3 mm in diameter, the necrotic parts were discarded and the explants transferred to fresh media. In the tabular enumerations which follow, the first transfers of callus tissue from the original inoculum are designated as primary subcultures. If these grew, any later subdivisions or transfers are referred to as secondary subcultures. Since secondary subcultures were transferred only as required, and at irregular intervals, they are not further designated. These secondary subcultures were maintained until they could no longer be transferred or until they were shown to be free of rust mycelium.

All photographs were taken at the stated magnifications and reduced one half in printing.

RESULTS

1. *Gymnosporangium juniperi-virginianae*. Development in callus culture.

TABLE II lists the cultural history of the 69 collections of telial galls from *Juniperus virginiana* used in this study. It includes data for the original axenic isolate (C-31-49) which has already been reported (22).[2]

Collections were made from localities along the Atlantic seaboard ranging from Massachusetts to Florida. The majority of collections were from the vicinity of New Haven, Connecticut, and Greensboro, North Carolina.

Galls of various ages were used for the initiation of callus cultures but, while galls were collected throughout the year, most of the successful cultures were obtained from maturing galls collected in August, September and October.

[2] Complete collection data for each locality are available in mimeographed form from the author on request.

An examination of the telial gall at any time during its development reveals intercellular mycelium of *Gymnosporangium* throughout the tissues. Reed and Crabill (**37**) have discussed the development and distribution of the mycelium in the gall as well as the appearance and function of the haustoria. Present observations are in complete accord with theirs. Sections cut from surface-sterilized galls show the mycelium, apparently undamaged, immediately below the suberized epidermis of the gall. Along the surface of fresh wounds the hyphae are frequently plasmolysed to a depth of several cell layers but in cutting the galls the mycelium is generally damaged to no greater extent than the host cells themselves. Apparently healthy mycelium can be found in gall tissues *in vitro* 5–6 months after initiation even though callus formation has not yet commenced.

TABLE II

HISTORY OF CULTURES FROM TELIAL GALLS OF GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE

No. of localities represented	No. of galls collected	No. of cultures started	Cultures contaminated after 7 days	Cultures contaminated after 11 days	Cultures contaminated after 60 days	No. of cultures failing to form callus but not contaminated	No. of cultures forming callus	No. of primary subcultures	No. of secondary subcultures	No. of *Gymnosporangium* strains isolated
54	8840	13,504	10,010	1740	360	1036	358	530	1729	7

The most important modification in the basic technique for the preparation of *Gymnosporangium* galls, from that employed with all other organs, was the pretreatment of the gall slices used in starting cultures with ascorbic acid. Hotson (**20, 21**) has commented on the necessity of suppressing the activity of terminal oxidases in these tissues prior to the induction of callus. The cut surfaces of the galls darken very rapidly upon exposure to air and in preliminary experiments it was soon obvious that callus formation never occurred at cut surfaces when melanin pigments had accumulated there. It has been indicated recently (**39, 42**) that polyphenol oxidase systems operating at cut surfaces may function to inhibit IAA or to destroy auxin diffusion. This would readily account for the failure of these oxidized gall tissues to regenerate callus. In all the experiments reported here the gall slices, after surface sterilization, were placed in 1% ascorbic acid in distilled water for 1 minute and were then planted on the respective media fortified with 500 mg/l ascorbic acid. In the presence of these concen-

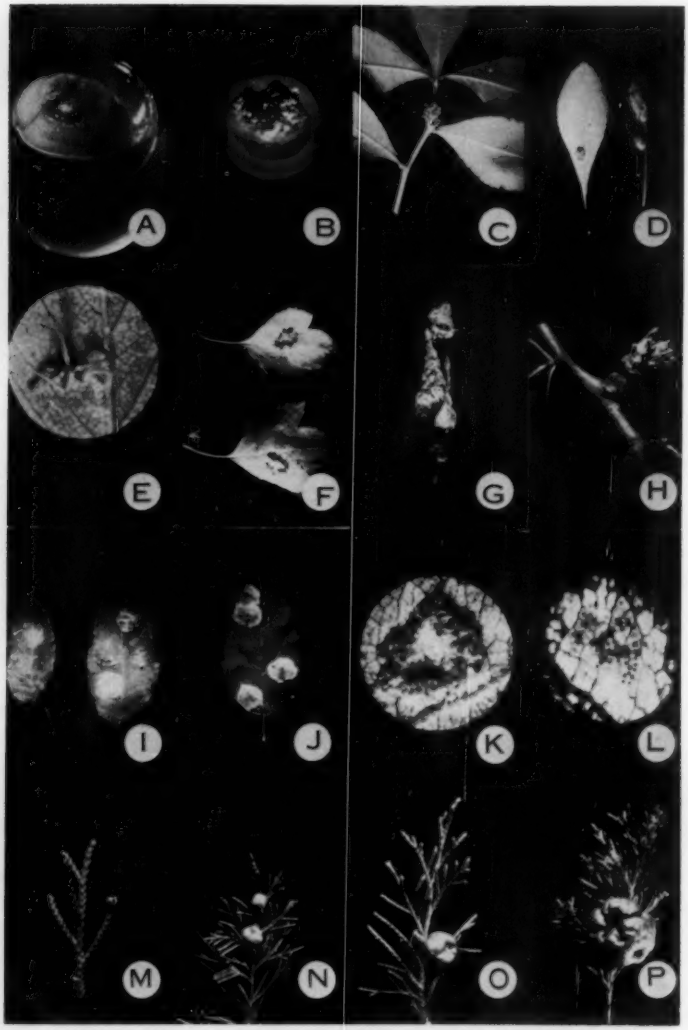

FIG. 2. Controlled inoculations with *Gymnosporangium juniperi-virginianae* (C-31-49). A. Isolated mycelial colony 60 days after transfer to Gautheret's from culture shown in Fig. 1, H. × 2. B. Uninfected cambial callus of *C. coccinea* used for testing. × 1. C. Stem gall on *C. Engelmanni;* field inoculation. × 0.5.

trations of ascorbic acid the cut tissues remained green or yellowish and there was no marked diffusion of oxidized pigments into the substrate. The continued presence of such high concentrations of ascorbic acid is undoubtedly inhibitory to callus formation as indicated by Henderson et al. (**18**) but its use is dictated by the necessity of preventing oxidative activity at the cut surfaces of these galls. The slow initial growth of gall calluses may have resulted from this inhibition but lower concentrations did not give adequate protection. Pretreatment of the gall slices for 10 minutes with 500 mg/l of glutathione or hydroquinone or of 100 mg/l of phenylthiourea or Na diethyldithiocarbamate in water prevented browning of the tissues but also inhibited callus formation.

In Hotson's experiments (**21**) on the development of callus tissue at the cut surfaces of the telial gall he reported growth stimulation upon addition to the basal medium of yeast extract, carrot juice and an aqueous extract of the gall tissues themselves, and noted toxicity from tomato, orange and lemon juices. He felt that this toxic effect might be due to increased sodium concentration introduced during titration of this acid medium to pH 6. In the extensive series of cultures carried out since that time all the additives used by Hotson and many more have been tried, but a definite toxicity has not been detected. On the other hand none of the various supplements, except coconut milk, have shown any marked stimulation of the callus tissues beyond that achieved on standard Gautheret's medium.

Initially it was assumed that galls in which the epidermis was intact would be relatively free from microorganisms, except for the mycelium of the rust, but it was shortly apparent that most of these galls harbored a rich flora of internal saprophytes and that these invading organisms would develop very rapidly on the culture medium once the gall was cut. As indicated in FIG. 4 the average contamination of cultures started from galls collected between September and May exceeded 80 per cent. In other words, most of the mature galls were invaded, and indeed by May, in these collections, all the galls were contaminated.

D. Aecia and pycnia on *C. Engelmanni*; greenhouse inoculation. × 0.5. E. Mature aecium on leaf of *C. crus-galli;* field inoculation. × 25. F. Aecia on *C. intricata;* greenhouse inoculation. × 1. G. Aecia on perennial stem gall, 14 mo. old, of *C. Engelmanni;* field inoculation. × 0.5. H. Dwarfed shoot on stem gall of *C. Engelmanni;* greenhouse inoculation. × 0.5. I. Pycnia on *P. coronaria;* detached leaf inoculation. × 1. J. Pycnia on *P. coronaria;* greenhouse inoculation. × 1. K–L. Aecia and pycnia on *P. coronaria;* field inoculation. × 20. M. Young gall developing on *Juniperus* in field 13 mo. after spot inoculation. N–O. Galls 14 mo. after field inoculation. P. Gall 17 mo. after field inoculation.

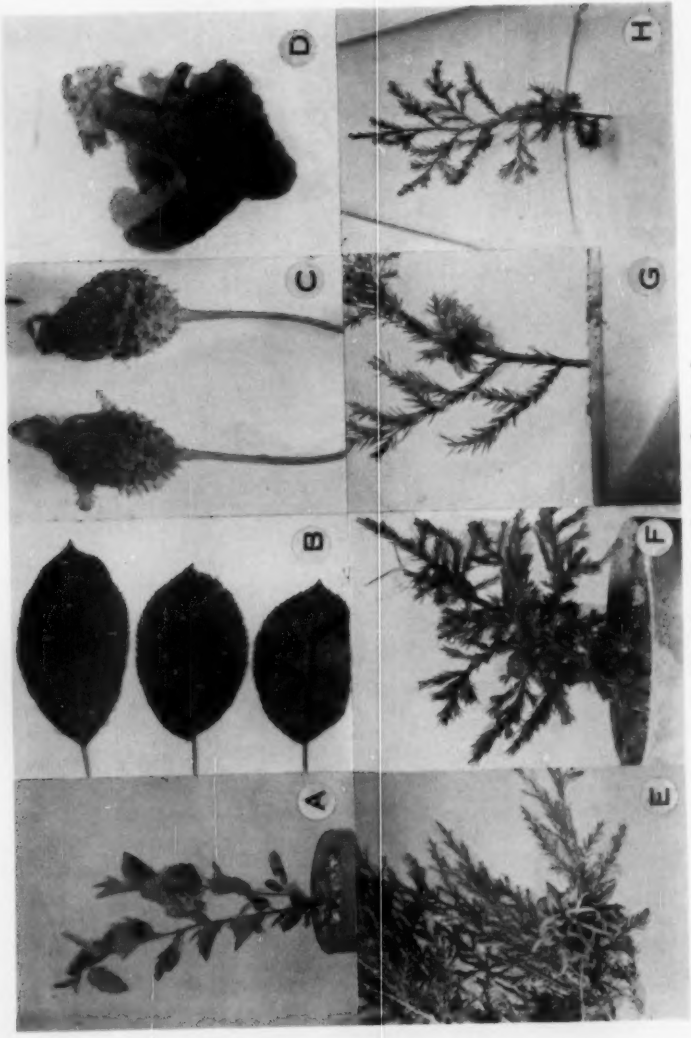

FIG. 3. (Legend on opposite page.)

This is not surprising since, because of insect ravages, very few galls reach maturity with an intact epidermis, and after the telia expand, invading organisms have an open pathway to the internal tissues of the gall. This probably explains why the galls become progressively more contaminated as they mature. To check the possibility that this high rate of contamination was the result of imperfect surface-sterilization 68 galls of various sizes with apparently undamaged epidermal surfaces were collected in October, immersed for 3 minutes in full-strength Clorox and rinsed in sterile water. They were then planted on Gautheret's agar and retained under the usual cultural conditions with three transfers to fresh media for a 9 month period without the appearance of a single contaminant. It was then obvious that surface sterilization was adequate and that the majority of the organisms which were appearing in our cultures were internal denizens of the galls which were not capable of growing out through the unbroken epidermis. One gathers the impression from the experiments of some tissue-culturists (**14**, **43**) that if sufficient care is taken to insure adequate surface sterilization and prevent superficial contamination the internal tissues of healthy plants with intact epidermal surfaces can usually be excised in a sterile condition. Keener, however, has provided data on the internal mycoflora of buds (**24**) which, along with the present study, indicate that many plant organs which are systemically infected with rust mycelium are also invaded by saprophytic organisms whether or not the epidermis is superficially intact.

TABLE II indicates that better than 90 per cent of the cultures were contaminated within 60 days of initiation. Internal contaminants in many cases continued to appear for 6–8 months after the cultures were started. Most of the cultures which remained uncontaminated ultimately developed callus but in many of the collections all the galls had saprophytic invaders. This in part explains the very large number of cultures started and the low rate of callus formation in them (FIG. 4). For this reason all cultures in which any microorganisms developed

FIG. 3. A. Pycnia of C-121-51 on seedling of *P. coronaria;* greenhouse inoculation. B. Pycnial flecks of C-121-51 on *P. Malus* var. Northern Spy; greenhouse inoculation. × 0.25. C. Aecia of C-31-49 on fruit of *C. Engelmanni;* field inoculation. × 1. D. Aecium of C-31-49, with peridium starting to split apically, on inoculated callus culture of *C. coccinea.* Portion of callus cut away to show aecium. × 60. E. Telial horns on gall, *22* mo. after field inoculation with C-31-49. F. Galls on seedling in greenhouse, *22* mo. after spray inoculation with C-31-49. G. Telial gall with expanding telial horns on seedling in greenhouse *23* mo. after carbowax spot inoculation with C-31-49. H. Telial gall with expanding horns on sterile seedling in *2* liter flask 12 mo. after carbowax spot inoculation with C-31-49.

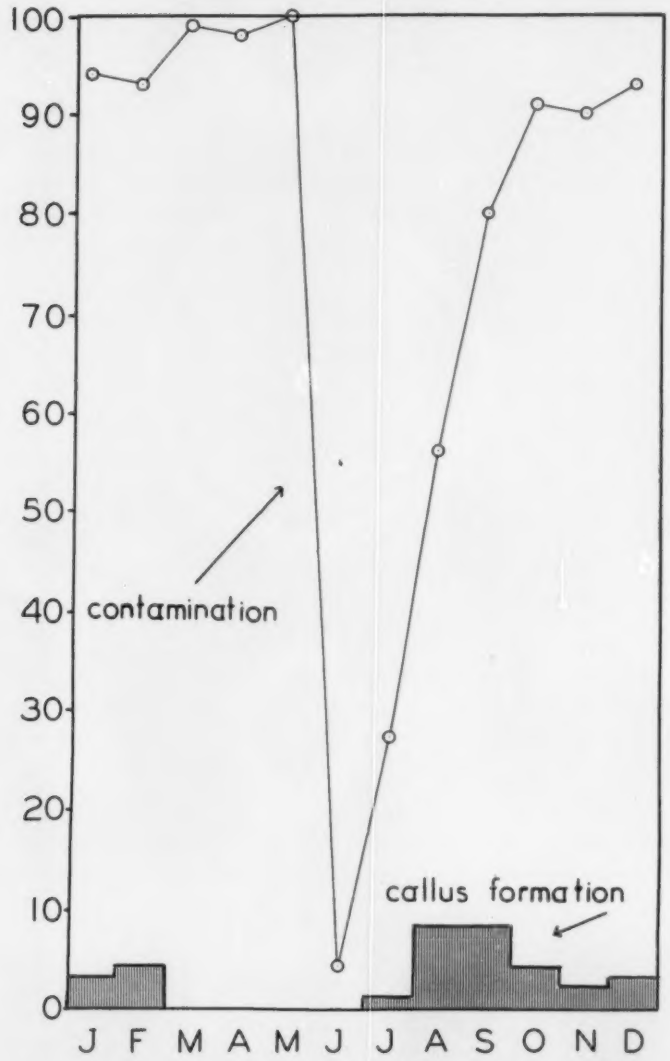

FIG. 4. *Gymnosporangium juniperi-virginianae*. Average per cent contamination of callus cultures with saprophytic fungi and bacteria, compared with callus formation by months in which the telial galls were collected. Total of 8840 telial galls from 54 localities represented. Collection and cultural data in TABLE II.

during the first 60 days of culture were routinely discarded, and these early contaminants were examined only in a cursory fashion.

The development of callus tissue on the gall sections is very irregular and its first appearance is not predictable. Visible callus has appeared upon the cut surface of the galls as soon as 2 weeks after planting but in other cases no callus was apparent until 5 months had elapsed. FIG. 4 also indicates the average per cent of callus formation by months in which the galls were collected. It will be recalled that galls collected in March and April are fully mature and may be producing teliospores, while those collected in May and June have already matured their teliospores and are senescent. In no case did galls collected during the latter period form callus. This may be attributed to the senescence of the tissue, but could as well relate to the fact that these mature galls are almost universally contaminated with extraneous fungi and bacteria. The rate of callus formation in superficially contaminated cultures has always been very low, and, when the growth of the contaminant was luxuriant, callus never appeared. However, there is evidence that the state of maturity of the gall tissues influences their regenerative ability quite aside from the question of the relation of contamination to callus formation.

Callus first appears as small, scattered, raised islands of thin-walled green parenchyma cells on the surface of the gall sections. These coalesce as growth proceeds and ultimately after 1–6 months the original tissue may be entirely blanketed by a thick layer of this callus (FIG. 1, B). The very rapid proliferation noted by Barker (**4**) in woody stems never occurred here. Voluminous masses of callus are produced while this growth is still attached to the original gall tissue, and when excised and transplanted the callus continues to proliferate indefinitely (FIG. 1, C) and may be transferred with ease. Regardless of the medium used the callus growth in subculture is slow, a typical callus explant doubling in weight after approximately 65 days on Gautheret's agar. Some of these calluses have been maintained by serial transfer for 8 years without perceptible change in rate of growth or appearance (FIG. 1 E). The structure of these rust-gall calluses is remarkably uniform and is not essentially different from that produced by cambial explants from uninfected branches and stems of *Juniperus* (FIG. 1, D). The callus grows as a lobulate mass of closely compacted parenchyma, interspersed with occasional nests of tracheids and patches of sclerenchyma. The color of the callus explants is variable and depends upon age and upon light intensity. Callus grown under 350 fc remains pale green for several months and then gradually becomes brownish and

encrusted on the surface. The same callus line under 890 fc develops patches of cells with brilliant red or purple, water-soluble pigments, presumably anthocyanins, within 20 days of initiation and continues to develop these pigmented areas throughout the growth period. Substitution of organic nitrogen sources, or the amino acids proline, hydroxyproline or serine, for the nitrates in Gautheret's medium also favors this pigment formation. However, the growth rate of the callus under 890 fc is much slower than at 350 fc and tissue proliferation is more irregular, the culture crumbling into small spherical masses. There was greater loss, upon transfer, of cultures grown at the higher light intensity. Callus grown continuously for 8 months without transfer at 890 fc was almost invariably dormant and incapable of continued growth, whereas the same material under 350 fc proliferated readily when transferred.

In an occasional culture started in the usual way there was no surface development of callus but the entire cube swelled externally as callus regenerated internally. Finally the dried cells at the surface ruptured and the internal callus proliferated through these fissures. Upon transfer these internal callus explants became indistinguishable from the usual surface explants.

In some callus lines certain cultures were noted which had apparently undergone habituation or anergy. These appeared during experiments in which, after callus had been grown on Gautheret's medium for a number of transfers, IAA and coconut milk were omitted from the medium in an attempt to inhibit the growth of the callus explants and thus force the systemic rust mycelium to become independent. Most of the explants did not survive under these conditions but some of them continued to grow in the absence of auxins, although their growth rate and appearance were quite different from those cultured in the usual way (FIG. 1, F). The habituated cultures were pale green in color, with rather smooth surfaces, and tended to spread uniformly over the agar rather than heap up into the characteristic piled calluses illustrated in FIG. 1, C. Aging habituated calluses became translucent and watery and could be transferred only by using a massive explant, since growth ceased in small explants immediately following transplantation. None of the habituated calluses survived more than five transfers in the absence of auxins, and the systemic mycelium did not survive, or become independent in any of them. In no case did all the cultures of any series show habituation following transfer to auxin-free media. Gautheret (**15**) has reviewed the studies on habituation and, as he points out, the factors responsible for this phenomenon are not well understood.

Upon callus formation the intercellular rust mycelium shortly in-

vades the new growth from the underlying tissues. It is interesting that this newly proliferated mycelium forms few and very minute haustoria in the young callus cells. Haustorial formation is restricted in young galls prior to the development of the telial sori (**37**) and this situation also obtains in young callus tissue. As the callus ages, particularly in explants which are drying on the surfaces, haustoria increase in number and size. Infected calluses, under these conditions, show practically every cell invaded by haustoria.

Following excision of the callus from the parental gall tissue and its transfer to fresh media the subsequent development of the rust mycelium is irregular. Its growth may keep pace with that of the callus explant, since in some lines calluses infected with rust mycelium have been successfully maintained for 8 years by periodic transfer. In many other cases, however, the mycelium fails to grow, or proliferates weakly, and the callus soon outgrows it and thereafter remains sterile. Some of these sterile calluses may have started from excised portions which were not yet invaded by mycelium, but in other instances sections of the callus taken after subculture reveal that the mycelium is confined to a limited region which may already be necrotic. A few instances have been noted where only a single lobe of a callus subculture was infected. Since the lobes tend to atrophy at the isthmus with the main colony and ultimately to be separated by many layers of necrotic tissue, these mycelial infections may become self limiting. In numerous cases, however, the mycelium has died out of healthy calluses after several subcultures, and this occurred regularly in all the habituated cultures examined. Mycelial survival was very poor in callus cultured under 890 fc but was close to 80 per cent in the calluses examined which were grown under 350 fc. This may relate to the better callus growth under lower light intensities. Since the primary objective of this study was to isolate the rust from the tissue, no healthy callus cultures which contained systemic mycelium were sacrificed and consequently the observations on mycelial survival in these cultures are neither sequential nor conclusive.

It has previously been shown (**22**) that when immature galls are sectioned and placed in culture the telial sori frequently complete their development, and produce functional teliospores. Hotson (**21**) has further described the situation which occurs when the developing telial horns in these callus cultures are removed aseptically. Shortly thereafter teliospores are produced directly from aerial mycelium which proliferates over the surface of the callus culture. The aerial hyphae have binucleate cells and in all respects, except the absence of haustoria, resemble the intercellular mycelium. Hotson's experiment was repeated

on a series of cultures from galls collected at Woodbridge, Connecticut, November 16, 1951. Eighteen of these cultures developed calluses over the cut surfaces of the cubes by March 1, 1952, and produced telial horns at the site of the telial primordia on the suberized surface of the original gall tissue. There was a small collar of white aerial hyphae around the base of each telial horn at this time. The horns themselves were approximately 15 mm long and the teliospore stalks were only partially gelatinized. The horns were severed with a sterile scalpel as close to their base as possible on March 8. Within four days the mycelial collar had spread as a thin subiculum over the callus swathing the sides of the blocks and on March 17 immature teliospores on short erect stalks were present on the surface of the callus. FIG. 1, M illustrates one of these isolated teliospores. Hotson (**21**), in his figures 2A and B, has illustrated the mycelial subiculum from the surface of the callus, and the present material appeared identical with his. The pedicels of the isolated teliospores never expanded as they do in the telial horns and the longest pedicel measured was only 19 μ in length. The teliospores measured approximately 14–25 by 7–9 μ, compared to average measurements of 35–50 by 12–19 μ for the teliospores in the horns. They were primarily two-celled but an occasional spore with only 1 cell was found. As far as could be determined these teliospores did not germinate *in situ*. On April 15 one callus was sacrificed and the aerial mycelium scraped off and placed in a hanging drop of Gautheret's solution in a van Tieghem cell. There were twelve teliospores in the drop. By April 17, 7 of the teliospores had produced promycelia and there were basidiospores present in the drop. The other 5 spores failed to germinate. In the remaining callus cultures the aerial mycelium never grew beyond the surface of the callus and did not become independent. In various other series of cultures where telial horns were formed it has been noted that a collar of aerial mycelium almost invariably appears around the base of the horn, covering the telial primordium and slowly spreading outward from it. In numerous attempts to lift this mycelium and transplant it to fresh media no growth has ever ensued.

The infected-callus subcultures were always carefully observed for the presence of any sori or rust spores. Out of the total of 2259 primary and secondary subcultures of *Gymnosporangium* which were carried for various periods of time, and the many subsequent transfers of these cultures, only 2 instances of sorus formation have occurred. One infected-callus culture, started from a gall collected at Salisbury, Maryland, January 9, 1952, and subdivided and transferred at approximately

3-month intervals, developed a single telial horn in December, 1954. The horn was 4 mm high and rose from one lobe of the callus. The spore pedicels did not gelatinize and the horn was left undisturbed until January 9, 1955, when the whole callus was killed, embedded in paraffin and sectioned. The entire callus lobe on which the horn appeared was invaded by hyphae with well developed haustoria. The telial primordium was somewhat diffuse and the host cells in the vicinity of the base of the horn were necrotic. No primordial disk, such as appears on the surface of the galls over the developing sori, was apparent. The teliospores in the horn were very closely compacted and did not appear mature and there was no evidence that any of them had germinated. The mycelium at the base of the sorus and in the surrounding callus was regularly binucleate (FIG. 1, J). This culture had been transferred to a fresh medium 8 days prior to the appearance of the telial horn and was growing under 350 fc at 20°.

The other instance of sorus formation occurred in a primary subculture of callus from a gall collected at Greensboro, N. C., on February 6, 1953. The primary subculture was made on April 9, at which time portions of callus clinging to the original gall slice were cut away and planted on fresh media. Growth continued and on June 4 the cultures were approximately 1 cm in diameter. At this time a very small telial horn protruded from one callus culture. It expanded to approximately 2 mm above the surface and then ceased to develop. It was excised and examined microscopically on June 11, at which time the teliospores were still immature (FIG. 1, N). When placed in water, very slight gelatinization of their stalks occurred. Well developed intercellular mycelium was present in a portion of the callus at that time. The remainder of the callus, which was allowed to continue growth, did not subsequently develop any sori. It is possible, in this instance, that when the primary explant was made a portion of the original gall containing a telial primordium was still attached to the transplanted callus and that this primordium continued growth. No other instances of telial sori have been noted in any of the callus subcultures. The appearance of these 2 telia on calluses was reminiscent of the aberrant telia described by Weimer (41) which he occasionally collected upon leaves of *Juniperus* infected with *G. globosum*, in that the telia were not accompanied by the characteristic hypertrophy of the underlying host tissue. It is curious that the presence of systemic mycelium in calluses does not incite there a hypertrophic reaction similar to that set up when a leaf or stem is infected, but in the many systemically in-

fected cultures of this and other rust species which have been examined no callus hypertrophy has ever been seen *in vitro*.

ISOLATION OF THE RUST FUNGUS

The circumstances leading to the first successful isolation of *Gymnosporangium juniperi-virginianae* in axenic culture have already been described (**22**). This strain (C-31-49) was isolated in February, 1950, and tested by reinoculation into tissue cultures of *Crataegus coccinea* L. between July 25 and September 25, 1950, at which time aecial sori had developed on the *Crataegus* callus cultures. Six additional strains, indistinguishable on morphological grounds from C-31-49, have since been isolated, using the same basic technique. Data concerning the isolation and testing of these isolates are compiled in TABLE III.

The growth of these fungi from the callus into the surrounding medium was always preceded by the same morphological changes in the callus. The emergence of the saprophytic rust mycelium was heralded by the appearance on the surface of the callus of dense pale-pink hyphae growing out between the surface cells in scattered patches (FIG. 1, G). For some days before the development of this aerial mycelium the growth rate of the affected calluses declined and they became very pale green with a translucent aspect. As the mycelium overgrew the surface and the patches coalesced the calluses turned brownish and became watery and soft. By the time the hyphae had grown into the agar medium, usually 30–40 days after their first appearance, the texture of the callus was so disrupted that it could no longer be lifted intact from the agar surface (FIG. 1, H). Cells from external portions of the calluses examined at that time were dead or dying and in many cases contained intracellular hyphae which had apparently invaded cells whose walls were ruptured. Other cells within the interior of the callus were still intact and contained rust haustoria. Ultimately the entire callus degenerated to a soft pasty mass. The fungus mycelium grew uniformly into the agar and upon transfer to fresh Gautheret's agar continued growing, independent of the host cells. The isolated mycelium on Gautheret's forms a very slow-growing tough cartilaginous colony, pink to pale tan during the first 60 days of growth, slowly changing to dark olivaceous (FIG. 2, A).

The nuclear behavior and growth of these 7 isolates on various media in axenic culture will be described in a subsequent publication. The nuclear condition of the strains at the time of isolation is listed in TABLE III. All 7 strains isolated in this manner have been repeatedly

TABLE III*

INOCULATION EXPERIMENTS IN FIELD, GREENHOUSE, AND TISSUE CULTURE WITH ISOLATED AXENIC CULTURES OF GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE

Isolate No.	Locality	Date gall collected	No. of callus subcultures	Date fungus isolated in axenic culture	Reinfection on *Crataegus*	Reinfection on *Pyrus*	Reinfection on *Juniperus*	Sori in tissue culture	Date culture lost	Nuclear condition of cells at isolation
C-31-49	Branford, Conn.	9/4/49	1	2/6/50	TC+ F+ G+	TC+ F+ G+	TC+ F+ G+ SS+	*Crataegus*+ *Pyrus*+ *Juniperus*+	still active 1958	binucleate
C-94-50	Woodbridge, Conn.	10/14/50	3	4/10/51	TC− F− G−	TC+ F+ G+	TC+ F+ G−	*Crataegus*− *Pyrus*+ *Juniperus*−	10/55	binucleate
NY-105-50	Cambridge, N. Y.	11/23/50	1	3/17/51	TC− F− G−	F+ G+	TC− F− G−	*Crataegus*− *Juniperus*−	9/52	uninucleate
C-121-51	Woodbridge, Conn.	1/29/51	from original callus	4/30/51	TC− F− G−	TC+ F+ G+	TC− F− G−	*Crataegus*− *Pyrus*+ *Juniperus*−	still active 1958	uninucleate
C-286-51	Woodbridge, Conn.	12/14/51	4	9/11/52	TC− F+ G−	TC− F+ G+	TC− F− G−	*Crataegus*− *Pyrus*− *Juniperus*−	10/55	uninucleate
NC-43-52	Thomasville, N. C.	11/15/52	1	4/10/53	TC− F− G−	TC+ F+ G+	TC+ F+ G+	*Crataegus*− *Pyrus*+ *Juniperus*−	10/55	binucleate
NC-219-54	Lexington, N. C.	10/1/54	2	5/19/55	TC− F− G−	TC− F+ G+	TC+ F+ G−	*Crataegus*− *Pyrus*− *Juniperus*+	still active 1958	binucleate

* Abbreviations: TC = tissue culture, F = field, G = greenhouse, SS = sterile seedlings.

subcultured on Gautheret's agar and many other media by periodic transfer of bits of the mycelium. The survival of these 7 strains in axenic culture and their ability to reinfect their respective hosts under various conditions are also listed in TABLE III. Three of the strains are still active, and the stock cultures of the 3 strains (C-94-50, C-286-51 and NC-43-52) which were lost in October, 1955, had been accidentally subjected to prolonged drying and high temperature in an incubator. It is felt that this treatment, rather than any inherent weakness of the cultures, contributed to their demise. On the other hand, strain NY-105-50 could not be revived after 18 months in culture, during which period it had been serially transferred 6 times on Gautheret's agar. The growth rate of this isolate was extremely slow, 2-month-old colonies measuring 3 mm in diameter, and its infective ability on the hosts was lower than any of the other isolates (TABLE III).

TISSUE-CULTURE INOCULATIONS

When isolated, the mycelium of our original axenic culture (C-31-49) had been shown to be composed of primarily binucleate cells (**21**, **22**), and to produce aecia but not pycnia on tissue cultures of *Crataegus*. Such a dikaryotic strain might be able to infect *Juniperus* as well as the rosaceous hosts, a possibility previously suggested by Olive (**33**) for the occasional dikaryotic basidiospores produced upon *G. clavipes*. Consequently, uninfected callus cultures of *Crataegus*, *Pyrus* and *Juniperus* were prepared to test the identity and infective capacity of the axenic isolates. In the tests run prior to 1953, callus of *Crataegus coccinea*, prepared by Gautheret's cambial technique (**13**) was used. Subsequent tests were done on callus of *C. Engelmanni*, *C. intricata* and *C. Youngii*. Callus of *Pyrus coronaria* was induced on young stem tips, and from immature fruits by sterilizing the entire fruit in Clorox, scoring the sides and thrusting the fruit partially below the surface of the agar medium. Callus developed on the wounds within 45 days and was excised and transferred in the usual way. This fruit callus was slow growing and formed a thin, tough periderm near the surface, which did not lend it to reinfection studies. Several attempts to induce callus from young twigs of various apple varieties, *P. malus*, were unsuccessful, but a limited amount of callus was obtained from green fruits of the variety Rome Beauty which is known to be susceptible to *G. juniperi-virginianae* (**37**).

Callus was prepared from uninfected branches of *Juniperus* in 1949 and 1950 and has been subcultured continuously since that time. In

TABLE IV

INOCULATIONS ON UNINFECTED CALLUS CULTURES OF VARIOUS HOST SPECIES WITH ISOLATED AXENIC CULTURES OF GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE

Isolate No.	Callus of host species	Date inoculated	No. of calluses inoculated	No. of calluses infected	Intercellular mycelium	Haustoria	Date first sorus noted	Type of sorus
C-31-49	*Crataegus coccinea*	7/25/50	12	11	+	+	9/25/50	aecia
C-31-49	*Crataegus coccinea*	12/10/51	20	14	+	+	3/2/52	aecia
C-31-49	*Juniperus virginiana*	12/10/51	20	20	+	+	—	none
C-31-49	*Crataegus intricata*	4/7/53	5	3	+	+	7/10/53	aecia
C-31-49	*Crataegus Engelmanni*	4/7/53	5	5	+	+	—	none
		11/6/54	5	5	+	+	3/7/55	aecia?
C-31-49	*Pyrus coronaria*	11/6/54	5	5	+	+	3/7/55	aecia
C-31-49	*Juniperus virginiana*	9/20/55	10	10	+	+	12/21/55	telial galls?
C-94-50	*Crataegus Engelmanni*	4/7/53	10	0	−	−	—	—
C-94-50	*Crataegus intricata*	4/7/53	7	0	−	−	—	—
C-94-50	*Crataegus Youngii*	10/10/54	15	0	−	−	—	—
C-94-50	*Pyrus coronaria*	10/10/54	4	2	+	+	1/6/55	pycnia
C-94-50	*Juniperus virginiana*	6/17/53	6	6	+	+	—	none
NY-105-50	*Crataegus coccinea*	12/10/51	10	0	−	−	—	—
	Juniperus virginiana	12/10/51	20	0	−	−	—	—
C-121-51	*Crataegus Engelmanni*	4/7/53	10	0	−	−	—	—
C-121-51	*Crataegus intricata*	4/7/53	10	0	−	−	—	—
C-121-51	*Crataegus Youngii*	1/10/55	5	0	−	−	—	—
C-121-51	*Pyrus coronaria*	10/10/54	7	5	+	+	12/19/54	pycnia
C-121-51	*Pyrus malus* var. Rome Beauty	3/15/55	10	9	+	+	4/30/55	pycnia
C-121-51	*Juniperus virginiana*	6/17/53	6	0	−	−	—	—

TABLE IV.—(*Continued*)

INOCULATIONS ON UNINFECTED CALLUS CULTURES OF VARIOUS HOST SPECIES WITH ISOLATED AXENIC CULTURES OF GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE

Isolate No.	Callus of host species	Date inoculated	No. of calluses inoculated	No. of calluses infected	Intercellular mycelium	Haustoria	Date first sorus noted	Type of sorus
C-286-51	*Crataegus Engelmanni*	4/7/53	9	0	−	−	—	—
C-286-51	*Crataegus intricata*	4/7/53	10	0	−	−	—	—
C-286-51	*Pyrus coronaria*	10/10/54	4	0	−	−	—	—
C-286-51	*Pyrus malus* var. Rome Beauty	3/15/55	10	0	−	−	—	—
C-286-51	*Juniperus virginiana*	6/17/53	6	0	−	−	—	—
NC-43-52	*Crataegus Engelmanni*	10/10/54	10	0	−	−	—	—
NC-43-52	*Crataegus intricata*	10/10/54	12	0	−	−	—	—
NC-43-52	*Pyrus coronaria*	10/10/54	5	4	+	+	12/3/54	pycnia, aecia
NC-43-52	*Pyrus malus* var. Rome Beauty	3/15/55	8	8	+	+	4/21/55	pycnia
NC-43-52	*Juniperus virginiana*	6/17/53	6	6	+	+	—	none
NC-219-54	*Crataegus Youngii*	7/2/55	10	0	−	−	—	—
NC-219-54	*Pyrus malus* var. Rome Beauty	7/2/55	4	0	−	−	—	—
NC-219-54	*Juniperus virginiana*	9/20/55	10	10	+	+	12/21/55	telial galls?

order to be certain that our testing material was not derived from hosts resistant to *Gymnosporangium* (**5**) the branches selected for the preparation of the callus were taken from trees bearing telial galls of this fungus. Before subculture these *Juniperus* calluses were carefully inspected for the presence of any intercellular mycelium of the fungus and none have shown any evidence of natural rust infection during the 9 years they have been in culture. Calluses used for testing were subdivided and transferred, 20 days before inoculation, to fresh Gautheret's medium to allow the cut surfaces to regenerate new cells. Only vigorous healthy callus tissue was used. All inoculations on sterile callus cultures of *Crataegus, Pyrus* and *Juniperus* were made with mycelium from 30-day-old agar cultures of the various isolated strains homogenized in Gautheret's liquid in the Waring Blendor. The inoculum was introduced directly onto the surface of the callus with a sterile hypodermic syringe and needle. The tubes were then sealed with Parafilm, and kept at 20° under 350 fc in a chamber at a relative humidity of approximately 56 per cent. Due to syneresis in the agar the relative humidity in the tubes during the early growth period was considerably higher than this.

The inoculations on callus cultures of the various host species are enumerated in TABLE IV. The inoculations made on *Pyrus coronaria* and on *P. malus* var. Rome Beauty were on fruit callus and the failure of strains C-286-51 and NC-219-54 to infect them may well have been caused by the thick periderm layer which develops in these calluses very soon after subdivision. Strain NY-105-50 did not infect any callus cultures, but its growth, as pointed out above, was very restricted in culture, and its identification as *G. juniperi-virginianae* rests only upon positive field and greenhouse inoculations. Strains C-94-50 and C-121-51 developed pycnia but no aecia on *Pyrus*. This was not unexpected, since the mycelial cells of these isolates were uninucleate. Strain NC-43-52, however, developed both pycnia and aecia on *P. coronaria* callus but only pycnia on *P. malus*. Again periderm formation may have prevented maturation of aecia. Cross spermatization of pycnia on callus cultures has not been attempted yet. Presumably the development of both pycnia and aecia in a sterile culture where cross spermatization does not occur would indicate that uninucleate and binucleate mycelia were both present in the inoculum.

The results of inoculations with the original strain C-31-49, which infected both *Crataegus* and *Pyrus*, again raise the question of the correct identification of this rust. Although all the aecia produced *in vitro* were smaller than in field infections, the structure of the aecia,

and particularly of the peridial cells, on callus of *Crataegus* and *Pyrus* was characteristic of that found on the respective hosts in the field. That is to say that infections on *Crataegus* callus fitted *G. globosum* whereas those on *Pyrus* callus were similar to *G. juniperi-virginianae*.

Because of the curious type of infection which strain C-31-49 caused in field and greenhouse inoculations on *Crataegus Engelmanni*, it was noteworthy that no aecia or other sori were formed on the callus cultures of this species by any of the remaining 6 isolates although the calluses were invaded by typical intercellular mycelium with haustoria in all cases. These inoculated calluses of *C. Engelmanni* were retained and transferred for 9 months following inoculation but no evidence of any sort of sporulation appeared. The invading mycelium remained in the callus and proliferated onto the agar only after 50 days growth and the callus tissue remained firm even after that period. The ability of C-31-49 to attack the resistant *C. Engelmanni* emphasizes the aberrant behavior of this strain.

With two exceptions (Strains C-31-49 and NC-219-54) none of the inoculations on *Juniperus* callus produced sori or spores, although all the binucleate strains formed intercellular hyphae bearing haustoria. The uninucleate strains produced small amounts of mycelium about the site of the inoculum, but this aerial mycelium did not grow into the deeper tissues of the callus and no haustoria were found in triturated material. The mycelium of these strains did not proliferate sufficiently to reach the agar surface. It should be borne in mind here that the inoculum was placed directly on the upper surface of the callus in order to prevent its prior spread on the medium. Where mycelium was first inoculated onto the agar surfaces it ultimately overgrew the callus culture but even in these cases the mycelium of the uninucleate strains did not form haustoria or deep infection. Infection is reported only when haustoria could be demonstrated, since all these strains can grow saprophytically over the surface of the calluses. It is recognized, of course, as Yarwood points out (**45**), that the ability of an organism to form haustoria with its host cells is not unequivocal proof that it is an obligate parasite, since many facultative parasites have this ability, but in this instance, where only a single organism was used in the inoculum, the development of typical haustoria is strong evidence that the organism was indeed *Gymnosporangium*.

In the two instances where sporulation occurred on the inoculated *Juniperus* callus small gall-like protuberances were formed over the surface after approximately 30 days. These swellings were very dense, purple-brown in color and were composed of callus cells with abundant

intercellular mycelium. Typical telial horns were not formed but numerous short-stalked teliospores developed between the callus cells. As far as could be determined there was no gelatinization of the telial stalks or of the sori. FIG. 1, L illustrates a callus cell with attached young teliospore and mycelium dissected from one of these rudimentary galls formed by C-31-49. In our cultures these teliospores never developed heavy walls but remained hyaline or pale brown, and none of them germinated.

FIELD INOCULATIONS

Inoculum for the first field tests was prepared by homogenizing mycelium from 30 day axenic agar cultures of the various isolated strains in Gautheret's in the Waring Blendor. The homogenate was then diluted with water and applied with an atomizer or concentrated and applied directly with a pipette to the leaves or branches. In a few cases mycelial homogenate was injected into young twigs with a syringe and 20 gauge needle, but no infection was ever secured in this way. All field inoculations in Connecticut on both cedar and rosaceous hosts were made with the mycelium suspended in Gautheret's fluid, but it was noted that liquid inoculum tended to roll off the leaves or to concentrate and dry out in spots before the mycelial fragments regenerated. The later inocula were therefore prepared by homogenizing the mycelium, concentrating the suspension in 15 ml centrifuge tubes, pouring off 10 ml of the supernatant and adding to the remaining 5 ml of concentrated homogenate a 1:1 mixture of "Carbowax" 1500W and "Carbowax" 6000 (Carbide and Carbon Chemicals Corp.) until a stiff paste was formed. This water-soluble "wax" is strongly hygroscopic and sufficiently adhesive to remain attached to plant surfaces for long periods of time. As far as could be determined, it is biologically inert and has no effect upon the regeneration and subsequent growth of the mycelium. In earlier experiments where the inoculum was suspended in lanolin and in petroleum jelly there was marked inhibition in the regeneration of the mycelial fragments. The Carbowax-paste inoculum was smeared thinly over the rosaceous leaves and young twigs, or was rubbed into the axils of the leaves of *Juniperus*. In all the later experiments with *Juniperus* a blob of the wax inoculum was simply pressed into a leaf axil and the mycelial strands soon grew out from the wax and invaded the dorsal surface of the leaf. This method of inoculation proved ideal, since it was advantageous to know the exact point at which the fungus was applied—a thing not possible with a sprayed inoculum. FIG. 2, C illustrates the development of aecia in a young leaf axil of *C. Engelmanni* following a spot inoculation with strain C-31-49 applied in Carbowax.

Plants in the greenhouse were either sprayed with the mycelial suspension *in toto* (FIG. 3, A and F) or spot-inoculated with Carbowax suspensions (FIG. 3, G).

The field inoculations in Connecticut were carried out with no other protection for the inoculated branches than Pliofilm bags, but the high spring and summer temperatures prevalent in North Carolina delayed or inhibited the regeneration of the mycelium and necessitated the use of additional devices to maintain high humidity and reduced temperature. The problem was solved by the use in the field of the so-called "iceless" refrigerator described by York (**46**). The celluloid refrigerator cones were placed on the branches immediately following inoculation and were retained in position for 2 weeks. They were then replaced with Pliofilm bags, which were left on until the rust sori or galls appeared. Detailed studies were not made on the temperature and humidity within these "iceless" refrigerators but two field readings taken on different days, when the ambient temperatures at branch level were 94° F and 78° F, gave respective readings within the refrigerators of 81° F and 69° F. These refrigerators were used in all spring and summer inoculations made in North Carolina.

The tests on rosaceous hosts in Connecticut were run on young trees (1–3 ft) of *Crataegus coccinea* and *C. crus-galli*, and on volunteer seedlings of an undetermined variety of *Pyrus malus* all growing wild in a pasture at Oxford, and on a mature tree of *P. malus* var. Jonathan at Bethany. Field tests after January, 1953, were carried out in North Carolina on various seedlings and small trees of *Crataegus Engelmanni*, *C. intricata*, *C. Youngii* and *Pyrus coronaria* growing wild at Sedgefield and on mature grafted stock of apples of the varieties Rome Beauty and Smith's Cider in an orchard at Lexington. These apple clones were known to be susceptible to *G. juniperi-virginianae* from observation of the same trees naturally infected the preceding year. All field inoculation were made on branches which had been covered with Pliofilm bags in February before the bud scales dehisced and prior to the emergence of telial sori on galls of *G. juniperi-virginianae* in the vicinity. These bags were removed briefly during inoculation and then replaced, tied, and left undisturbed until pycnia or aecia appeared on the developing leaves. Where only pycnia appeared cross spermatization was attempted by mixing pycnial nectar with a fine-pointed camel's hair brush, after which the bags were replaced. The high spring temperatures encountered in North Carolina caused some leaf burning inside these bags, which may have interfered with the optimum development of the rust, and further tests were therefore run in the greenhouse.

Field tests were run on *Juniperus* trees 2–4 ft tall growing under natural conditions in Connecticut and North Carolina. Since Berg (**5**) has indicated that some cedars may be naturally resistant to *G. juniperi-virginianae*, trees were selected which had borne galls from natural infections during the previous year. Any galls present on the trees at the time of testing were removed by clipping the infected branches. Inoculations made on cedar between June and October, when aeciospores from naturally infected rosaceous plants in the vicinity might be present, were carried out on branches which had been bagged in late May before aecia were mature on the rosaceous hosts. After inoculation the bags were loosely tied at the base of the branches and left in place until December, when they were removed and the inoculated branches tagged and left unprotected until the young galls appeared the following summer. It should be remembered that it is not possible in the field to detect *Gymnosporangium* infections for approximately one year following the fall of aeciospores onto the cedar leaves. Hence the removal of visible galls from cedar branches in the spring does not guarantee that these branches do not bear leaves infected by aeciospores of the preceding season which have not yet developed visible galls. However, these young galls are usually detectable by July or August of that year and for this reason the occasional galls which appeared on our inoculated branches during the first summer following inoculation were removed and ignored in recording the infections.

The results of field inoculations with axenic cultures of C-31-49 are presented in TABLE V. Infections following field inoculations with the remaining 6 axenic isolates are summarized in TABLE III. Five species of *Crataegus* as well as *Pyrus coronaria* and *P. malus* were infected by C-31-49. *C. Engelmanni* was primarily attacked on the young stems by this strain (FIG. 2, C, G, H), with only sporadic leaf infections. This immediately raised the question whether the stem galls which developed on this species after inoculation might not, in actuality, be natural, perennial, systemic infections caused by *Gymnosporangium clavipes*, which parasitizes *C. Engelmanni* heavily in this section of North Carolina. This appeared to be a distinct possibility since not all the inoculated trees became infected and the caulicolous position of the aecia coupled with the appearance of the peridial cells were all reminescent of *G. clavipes*. Nevertheless these trees had been protected by bagging during the spring preceding inoculation, when basidiospores of *G. clavipes* were disseminated, and aecia had appeared on the *Crataegus* stems only where spot inoculations had been placed. While *G. clavipes* is not known to form systemic or perennial mycelium in its

TABLE V

FIELD INOCULATIONS CARRIED OUT WITH GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE, STRAIN C-31-49, IN CONNECTICUT AND NORTH CAROLINA. ALL HOSTS GROWING WILD UNDER NATURAL CONDITIONS

Locality	Date of inoculation	Host species	No. of trees inoculated	Type of inoculum	No. of trees infected	No. of sori produced	Type of infection produced	Date infection noted
Oxford, Conn.	7/15/50	*Juniperus virginiana*	12	liquid	2	7	telial galls	8/7/51
Oxford, Conn.	8/20/50	*Juniperus virginiana*	14	liquid	1	4	telial galls	8/7/51
Oxford, Conn.	9/7/50	*Juniperus virginiana*	10	lanolin	0	0	—	—
Oxford, Conn.	9/7/50	*Juniperus virginiana*	4	petroleum jelly	0	0	—	—
Oxford, Conn.	5/19/51	*Crataegus coccinea*	4	liquid	1	12	aecia	7/4/51
Oxford, Conn.	5/19/51	*Crataegus crus-galli*	6	liquid	1	4	aecia	7/4/51
Oxford, Conn.	6/4/51	*Pyrus malus* var.?	3	liquid	2	many	pycnia, aecia	7/28/51
Oxford, Conn.	5/12/52	*Crataegus coccinea*	6	liquid	0	0	—	—
Oxford, Conn.	5/12/52	*Crataegus crus-galli*	8	liquid	4	19	aecia	7/24/52
Bethany, Conn.	6/1/52	*Pyrus malus* var. Jonathan	1	liquid	1	many	pycnia, aecia	7/14/52
Sedgefield, N. C.	11/24/52	*Juniperus virginiana*	15	liquid	0	0	—	—
Sedgefield, N. C.	12/14/52	*Juniperus virginiana*	5	carbowax	0	0	—	—
Sedgefield, N. C.	2/10/53	*Juniperus virginiana*	5	carbowax	0	0	—	—
Sedgefield, N. C.	4/1/53	*Juniperus virginiana*	12	carbowax	0	0	—	—
Sedgefield, N. C.	5/10/53	*Juniperus virginiana*	3	carbowax	0	0	—	—
Sedgefield, N. C.	6/24/53	*Juniperus virginiana*	4	carbowax	2	14	telial galls	7/2/54
Sedgefield, N. C.	7/19/53	*Juniperus virginiana*	4	carbowax	4	38	telial galls	7/2/54
Sedgefield, N. C.	8/20/53	*Juniperus virginiana*	6	carbowax	6	54	telial galls	7/2/54
Sedgefield, N. C.	10/4/53	*Juniperus virginiana*	4	carbowax	3	10	telial galls	8/14/54

TABLE V.—(*Continued*)

FIELD INOCULATIONS CARRIED OUT WITH GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE, STRAIN C-31-49, IN CONNECTICUT AND NORTH CAROLINA. ALL HOSTS GROWING WILD UNDER NATURAL CONDITIONS

Locality	Date of inoculation	Host species	No. of trees inoculated	Type of inoculum	No. of infected trees	No. of sori produced	Type of infection produced	Date infection noted
Sedgefield, N. C.	11/27/53	*Juniperus virginiana*	4	carbowax	1	1	telial galls	8/14/54
Sedgefield, N. C.	1/7/54	*Juniperus virginiana*	3	carbowax	0	0	—	—
Sedgefield, N. C.	5/20/54	*Juniperus virginiana*	4	carbowax	3	12	telial galls	7/19/55
Sedgefield, N. C.	7/10/54	*Juniperus virginiana*	8	carbowax	8	217	telial galls	7/28/55
Sedgefield, N. C.	4/6/53	*Crataegus intricata*	2	carbowax	1	4	pycnia, aecia	6/29/53
Sedgefield, N. C.	5/10/53	*Crataegus Youngii*	4	carbowax	0	0	pycnial flecks	—
Sedgefield, N. C.	5/10/53	*Crataegus Engelmanni*	6	carbowax	2	19	aecia (on stems)	8/17/53
Sedgefield, N. C.	5/10/53	*Pyrus coronaria*	3	carbowax	3	many	pycnia, aecia	7/3/53
Sedgefield, N. C.	6/11/53	*Pyrus coronaria*	4	carbowax	4	many	pycnia, aecia	7/14/53
Sedgefield, N. C.	5/19/54	*Pyrus coronaria*	4	carbowax	4	many	pycnia, aecia	6/13/53
Sedgefield, N. C.	5/19/54	*Crataegus Engelmanni*	4	carbowax	1	7	aecia (on stems)	7/29/54
Lexington, N. C.	5/21/54	*Pyrus malus* var. Rome Beauty	1	carbowax	1	26	pycnia	7/3/54
Lexington, N. C.	5/21/54	*Pyrus malus* var. Smith's cider	1	carbowax	1	many	pycnia, aecia	7/3/54
Sedgefield, N. C.	5/28/55	*Crataegus Engelmanni* (young fruits)	3	carbowax	3	many	aecia (on fruits)	8/23/55

rosaceous hosts, very large stem galls of *G. clavipes*, with much secondary thickening, have been noted on this species of *Crataegus* which are indistinguishable from that illustrated in FIG. 2, G. These galls appear to have overwintered and proliferated the following summer.

In view of the possibility that this rust might be systemic in *C. Engelmanni* in the field these inoculations were repeated the following year with practically identical results. Only 1 tree in 4 inoculated developed aecia and here again there was no sign of infection except at the points of inoculation. As a control 6 small trees in the vicinity of the inoculated clones were also bagged but were not inoculated and none of these showed infection with *G. clavipes*. As a further check on this situation flowering branches on 3 trees of *C. Engelmanni* were bagged in early May before the buds opened and the flowers were allowed to mature in the bags. At anthesis the flowers were cross pollinated and rebagged. When the petals had fallen on May 28, 16 young pomes were spot-inoculated on the sepal ends with a Carbowax suspension of mycelium of C-31-49 and the bags replaced. Nine of these fruits reached maturity in August and eight of them developed aecia on the surface (FIG. 3, C). The aecial structure and shape of the peridial cells were characteristic of *G. clavipes* and the aeciospore measurements corresponded with those given by Arthur (3) for this species. No pycnia were noted on these fruits as they developed. No other infections of *G. clavipes* developed on the unbagged fruit of the inoculated trees. Since the results of greenhouse tests (TABLE VI) bear out the field studies, it appears that clones of *C. Engelmanni* are differentially susceptible to infection with strain C-31-49. The suggestion that this strain C-31-49 might actually be *G. clavipes* is effectively ruled out by the fact that it produced globose foliar galls when inoculated on *Juniperus* as described below. The problem of systemic infection of *Crataegus Engelmanni* by *G. clavipes* remains to be worked out, as does the marked similarity of the aecia produced by C-31-49 on *C. Engelmanni* to those described for *G. clavipes*.

A point of particular interest brought out by the field studies is the fact that well developed pycnia have not been noted on any of the infected clones of *Crataegus* except *C. intricata*, while they have been present on all infections produced on *Pyrus*. In our earlier report (22) we noted the absence of pycnia upon *Crataegus* tissue cultures inoculated with strain C-31-49, and speculated that this might relate to the fact that the isolated mycelium was binucleate and presumably dikaryotic and hence would be capable of initiating aecia directly, since dikaryotization had already occurred. A somewhat similar situation is known to occur in several species of *Cronartium* where aeciospores are capable of

reinfecting *Pinus* and repeating the aecial stage without pycnial formation (Meinecke, 28). This peculiar behavior was found only in our strain C-31-49, since the other binucleate strains did not infect *Crataegus*. The uninucleate strains all produced *pycnia* on *Pyrus* and did not infect *Crataegus* with the exception of C-286-51. This strain produced a few pycnial flecks on *C. intricata* in North Carolina but aecia did not mature on the infected leaves.

Another point brought out in TABLE V requires comment. In general, the results of inoculations in Connecticut were much less consistent

TABLE VI

GREENHOUSE TESTS CARRIED OUT WITH GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE STRAIN C-31-49 AT GREENSBORO, N. C. TEMPERATURES CITED ARE AT THE TIME OF INOCULATION IN "ICELESS" REFRIGERATOR

	Temperature (C)	Host species	No. of plants inoculated	Type of inoculation	No. of infections	Type of infection	Date infection noted
2/10/53	16°	*Juniperus virginiana*	3	liquid	10	telial galls	5/27/54
4/18/53	19°	*Juniperus virginiana*	4	liquid	26	telial galls	5/27/54
6/12/53	25°	*Juniperus virginiana*	10	carbowax	41	telial galls	5/27/54
4/18/53	19°	*Crataegus Engelmanni*	2	carbowax	7	aecia (on stems)	8/4/53
4/18/53	19°	*Crataegus intricata*	4	carbowax	17	pycnia, aecia	5/21/53
4/18/53	19°	*Pyrus coronaria*	4	carbowax	many	pycnia, aecia	5/21/53
7/4/54	27°	*Juniperus virginiana*	6	carbowax	58	telial galls	6/23/55
5/12/54	20°	*Pyrus malus* var. Jonathan	2	carbowax	many	pycnia, aecia	6/18/54
5/12/54	20°	var. Rome Beauty	2	carbowax	41	pycnia, aecia	6/18/54
5/12/54	20°	var. Northern Spy	2	carbowax	12	pycnia	6/24/54
5/12/54	20°	var. Smith's Cider	2	carbowax	many	pycnia, aecia	6/24/54
4/29/55	18°	*Pyrus coronaria*	6	liquid	many	pycnia, aecia	6/10/55
5/4/55	22°	*Crataegus Engelmanni*	3	carbowax	5	aecia	7/28/55

than in North Carolina and the infectivity of the isolated fungus was much lower. However, most of the inoculations in Connecticut were carried out with liquid inoculum sprayed on the trees while those in North Carolina were made with inoculum suspended in Carbowax and spotted. The second method of inoculation is much superior, since the inoculum remains in intimate contact with the host and does not become desiccated under field conditions. Inocula suspended in Lanolin and

Petroleum jelly never caused infection. Therefore these results are probably not comparable in judging the infectivity of this strain.

The seasonal susceptibility of *Juniperus* to infection is interesting. Inoculations carried out on cedar from May to November usually resulted in infection, whereas trees inoculated in other months were resistant. It will be recalled that new growth develops on cedar during the summer and early fall and apparently the mycelium can invade only the new leaves. The maturation of telial galls on the trees tested in Connecticut was not followed to sporulation because the author moved to North Carolina in the fall of 1952. Hence the records on *Juniperus* in Connecticut are complete only through one year and refer to the formation of young galls. In North Carolina, however, the entire developmental cycle was recorded. Many of the young galls appearing on inoculated trees during the summer of 1954 failed to mature as a result of the prolonged drouth in August and September of that year. These galls reached 0.5–1 cm in diameter by August but then shriveled and fell off the branches. A few galls on trees in more favored localities matured and produced telia in the spring of 1955. In contrast, almost 100 per cent of the galls appearing during the wet summer of 1955 matured in the spring of 1956 and produced luxuriant crops of telia (FIG. 3, E). Experiments are now in progress on the reisolation of the fungus from galls produced by inoculation of cedar with axenic isolates, by the usual tissue-culture technique. It will be of great interest to know whether the fungus in these galls retains its saprophytic ability, or whether passage through the host has resulted in reversion to strict obligate parasitism.

GREENHOUSE INOCULATIONS

Because of the uncertainty in field inoculations, induced by weather fluctuations over the long period required for the galls to mature, 2–3-year-old cedar trees were dug in the field, potted and removed to a cool greenhouse. Many of these seedlings were still in juvenile leaf (FIG. 3, F, G) and of course nothing was known about their natural susceptibility or resistance (**5**). These seedlings were continuously protected in the greenhouse under a tent of Pliofilm sheeting for one full year prior to inoculation and any galls which developed on them during this period were removed. They were then inoculated and again protected for the duration of the experiment. Field-collected seedlings of *Crataegus* and of *Pyrus coronaria* were potted in January and February and removed to the same greenhouse, where they were protected under tents of Pliofilm until inoculation. In addition to the native species, 1-year-

old grafted plants of the apple varieties Northern Spy, Rome Beauty, Jonathan and Smith's Cider obtained from the nurseries of Stark Bros., Louisiana, Mo., were potted and tested in the greenhouse. Since the only available greenhouse was also used for commercial flower production, close control on temperature and humidity was impossible and the seedlings of *Juniperus* did not grow well under these conditions.

Inoculum was prepared in Carbowax as already described and Hunt's modification (**23**) of the "iceless refrigerator" was used on the branches for 2 weeks following inoculation.

TABLE VI summarizes the greenhouse inoculations carried out with C-31-49. In general, these substantiated the field experiments. Inoculations on *Juniperus* in April, June and July resulted in infection and gall formation, whereas those made in February, before new growth had started on the transplanted seedlings, produced no galls. Thus inoculated new growth on cedar became infected but older leaves which had hardened off were resistant. In spite of unfavorable conditions several of the inoculated *Juniperus* seedlings developed mature telial galls which ultimately produced telia (FIG. 3, F, G). Inoculations on the young leaves and stems of the rosaceous species were successful (FIG. 2, D, F, H, J; FIG. 3, A, B) and in the majority of cases the aecia matured and formed aeciospores. The apple varieties used are all reported as susceptible (**6**, **29**, **37**), but in our tests inoculations on the variety Northern Spy produced only pycnial flecks and aecia never developed in the greenhouse (FIG. 3, B). However, McNew (**27**) has indicated that differential susceptibility to infection by *G. juniperi-virginianae* is widespread amongst apple varieties. Since all these tests were carried out under closed tents, and the plants were more or less protected from insects, cross spermatization by mixing pycnial exudate on the leaves with a camel's-hair brush was employed. No attempt was made to mix the nectar aseptically.

As in the case of field inoculations with C-31-49, the greenhouse tests on *C. Engelmanni* resulted primarily in stem infections, although an occasional leaf infection was secured (FIG. 2, D), while infection on *C. intricata* was confined to the leaves. None of the other 6 axenic strains of *Gymnosporangium* infected *Crataegus* in the greenhouse (TABLE III).

INOCULATIONS ON DETACHED LEAVES AND STERILE SEEDLINGS

Infectivity tests were also made with strains C-31-49 and C-121-51, using detached leaves of *Pyrus coronaria* and *P. Malus* var. Rome

Beauty floated on Gautheret's solution and on 6% sucrose in water. Young leaves were forced on field-collected twigs, cut Feb. 3, 1954, by placing them at 20° under 350 fc. When the expanding leaves reached a length of 2.5–4 cm they were detached and floated dorsal surface down with petioles submerged. Mycelial homogenate in water was applied in discrete droplets to the ventral surface. Pycnial infections developed on *P. coronaria* with both strains 23 days after inoculation (FIG. 2, I). Although the culture fluid was replaced every five days, the cultures became contaminated with saprophytes and aecia did not mature. However, aecial primordia were present on the leaves infected with strain C-31-49 when the cultures were discarded. Pycnial flecks and some aerial hyphae developed at the site of inoculation on Rome Beauty leaves with both strains but the pycnia did not mature.

In an attempt to obviate the contamination which occurred in the floating-leaf cultures dormant twigs of *P. coronaria* and *Crataegus intricata* were cut March 12, 1954, and forced in water under large bell jars. The expanding leaves were sprayed March 28 with a mycelial homogenate of the same strains and then held under 890 fc at 20° for 45 days. The leaves under these conditions never expanded to normal size and were yellowing in approximately 32 days. Both strains developed pycnia on *P. coronaria* by April 24, but pycnial exudate was not secreted and in spite of attempts at cross spermatization, no aecia developed. Pycnial flecks were present by April 30 on 14 out of 39 leaves of *C. intricata* sprayed with C-31-49 but they did not mature and no flecks developed on the leaves sprayed with C-121-51.

Finally, to remove any possible doubt about the sterility and freedom from systemic infection of the test plants, sterile-seedling cultures of *Juniperus* were prepared. Mature seeds were surface-sterilized and the embryos excised aseptically and grown on Gautheret's agar with 20 μg/l of IAA. These embryos were started in shell vials at 20° under 350 fc continuous illumination and were transferred after 5 months to fresh medium in 2 liter sealed flasks.

Eleven sterile seedlings were spot-inoculated aseptically in young leaf axils with a carbowax suspension of C-31-49 on October 10, 1954, when they were 14 months old and approximately 4 inches tall. They were kept at 20° under 350 fc until February 20, 1955, and were then moved to a greenhouse (approximately 70° F) and maintained there until February, 1956. Three cultures became contaminated with *Aspergillus*, the agar was overgrown and the seedlings finally died. A very minute telial gall was noted on one seedling on March 21, 1955. By April 27, 4 additional cultures had developed galls which by September 15, 1955,

showed telial primordia. One culture was then opened and sterile water sprinkled on the gall and seedling. Telial horns were extruded within 2 hours (FIG. 3, H). The galls were characteristic of *G. juniperi-virginianae* in all respects except size, none of them exceeding 1 cm in diameter. When the cultures were discarded in February, 1956, teliospores were present in all the galls, but the horns had not extruded, since, by this time, the cultures were partially desiccated. The three cultures which never developed any sign of infection presumably contained resistant seedlings. The maturation of telial galls in these cultures in approximately 11 months, in contrast to the usual developmental period of 18–20 months in the field, is probably correlated with the fact that infection took place immediately after inoculation and, under greenhouse conditions, the sterile seedlings grew continuously without entering the usual dormant periods.

Several attempts were made to grow sterile seedlings of *Crataegus* and *Pyrus* by Nickell's technique (**32**) but these were unsuccessful. The cotyledons expanded but the embryos failed to root and finally died. We have not yet been able to carry out test inoculations of the rosaceous host under completely aseptic conditions.

2. *Gymnosporangium globosum* Farl.

Telial galls of this species were collected at Lexington, North Carolina, on November 12, 1953, and 63 cultures started. The galls of *G. globosum* are much smaller than those of *G. juniperi-virginianae* and it is correspondingly more difficult to obtain uncontaminated gall parenchyma to initiate cultures. Only 2 galls produced callus and the subcultures survived only one transfer. The appearance of the callus was indistinguishable from that produced on the telial galls of *G. juniperi-virginianae* under similar conditions but no rust mycelium developed in it. The telial horns never expanded on the gall slices *in vitro* and aerial mycelium was not produced. Sections through a gall slice one month after the cultures were initiated showed that the intercellular mycelium was plasmolysed and probably dead.

Pycnial and aecial infections by this species on *Crataegus* fruits are separated with great difficulty from similar infections of the same host by *G. clavipes*. The material used here was so identified by the smaller chestnut-colored aeciospores and the sculpturing of the peridial cell walls (**25**). In the North Carolina material of these two species which has been examined, particularly on fruits and stem galls, there is considerable doubt about the significance of these characters in establishing a valid

identification. The measurements overlap and the colors of the aeciospores are variable in sori of different ages.

Callus was induced on 2 fruits and on 4 stem galls of *Crataegus Engelmanni* infected with *G. globosum* by the technique described above. Intercellular mycelium did not proliferate into any of these calluses, and the primary subcultures were completely sterile.

3. *Gymnosporangium clavipes* (Cke. et Pk.) Cke. et Pk.

The aecial stage of this rust has been collected on young fruits of *Amelanchier canadensis, Pyrus arbutifolia, Crataegus Phaenopyrum* and *C. Engelmanni* from a number of localities in Connecticut and North Carolina. The telial stage is abundant on *Juniperus virginiana* in North Carolina. Numerous attempts were made to induce callus on young infected fruits in which pycnia and aecia were developing by surface-sterilizing the intact fruit, scoring the epidermis and thrusting the fruit below the surface of the nutrient medium. These attempts were uniformly unsuccessful since all the infected fruits were contaminated. Stem galls of this species on *Crataegus* were surface-sterilized and cut into rounds from which the periderm and cortex were stripped. Sections of these rounds containing cambium were then cut out and planted, but again they were totally contaminated.

Callus was induced by the same technique on infected branches of *Juniperus* collected in February before telia had expanded. A majority of the cultures were contaminated and, in those which formed callus, there was no development of intercellular mycelium in the new growth. Dodge (11) has shown that the telial mycelium of this species is largely confined to the external layers of the periderm and cortex of the *Juniperus* stem. The superficial fungal mycelium was undoubtedly damaged by the sterilizing agents and since the callus regenerated from the cambium, which presumably was not invaded by systemic mycelium, the fungus never proliferated. The same difficulty was experienced with cambial cultures of *Juniperus communis* infected with *Gymnosporangium clavariaeforme*.

DISCUSSION

In light of the results of these inoculations the identity of strain C-31-49 requires further elucidation. This strain is unusual in its ability to infect *Crataegus* as well as the expected host, *Pyrus,* and even more unusual in that the aecial fructifications on *Crataegus* resemble respectively the hawthorn rust, *G. globosum,* on *C. coccinea, C. crus-galli, C. Youngii* and *C. intricata* and the quince rust, *G. clavipes,* on

C. Engelmanni. However, it failed to infect callus cultures of *C. Engelmanni*. On the several varieties of *Pyrus* the aecia agree well with those reported for *G. juniperi-virginianae* and the telial galls and telia on *Juniperus* are consistently characteristic of this species. The primary reason for assigning this isolate to *G. juniperi-virginianae* in the first report (**22**) was the fact that great care was taken in selecting galls of this species from cedar to avoid the accidental collection of *C. globosum*. However, it must be admitted that it is very difficult to separate the immature galls of these species, particularly in the winter condition, and galls of *C. globosum* might have been inadvertently collected.

The other 6 axenic isolates behaved normally by infecting *Pyrus* and failing to infect *Crataegus*, except C-286-51, which did produce pycnial flecks but no aecia on *C. intricata* in field inoculations. This isolate failed to infect *C. intricata* or other species of *Crataegus* in greenhouse or tissue-culture inoculations.

Several alternative explanations occur for the situation found in C-31-49: 1) This strain represents a mixed culture of *C. globosum* and *G. juniperi-virginianae*. 2) The inoculation techniques were not sufficiently controlled to exclude contamination by wind-borne spores of one or the other of the species of *Gymnosporangium*. 3) The natural host specificity of *G. juniperi-virginianae* is less strict than has been assumed (**26**) and some strains may be able to infect *Crataegus*. 4) The structure of aecia, peridial cells and aeciospores (**25**) is mediated by the particular host rather than by the fungus, and *G. juniperi-virginianae* might thus produce characteristically different aecia on different host species. 5) Axenic culture has so modified the genotype of this strain that its host specificity has been broadened.

Considering the method used to isolate this strain, which involved culturing single gall slices in separate tubes, the first alternative would involve the assumption that we were dealing with a compound gall of the 2 species, an inference requiring an incredible extension of circumstances. The precautions taken to avoid accidental spore contamination during inoculation appear adequate and are certainly as rigid as those ordinarily employed by competent uredinologists in field and greenhouse testing of rust races. Furthermore the results reported have been consistent, upon repetition, which would not be anticipated were accidental contamination at the base of this apparent discrepancy. The explanation probably resides in some combination of the latter 3 alternatives. No carefully controlled recent study of the infective capacities of these 3 species of *Gymnosporangium* upon various wild hosts in different areas is available and the host specificity of *Gymnosporangium juniperi-*

virginianae may well prove more plastic than present information would indicate.[3] This is admittedly a speculation but it seems more plausible than to assume that a mixed culture of two species of *Gymnosporangium* has been isolated. Since *G. globosum* is known to attack *Pyrus Malus* particularly in the northeast (**29**) and since *G. juniperi-virginianae* may attack *Crataegus* (**34**, **6**) this hypothesis is subject to test. Pammel (**34**) has reported infections on *Crataegus* by *G. juniperi-virginianae* but these have been regarded (**6**) as misidentifications, since his description of the aecia produced on *Crataegus* fits those of *G. globosum*. A detailed description of the aecia of *G. globosum* as they occur on apple is not available, although Miller (**30**) has noted that the peridial tubes are persistent under these conditions, and his studies on *G. globosum* have indicated (**29**) that its infective ability varies with the range of its hosts. In any case a positive identification of this culture on the evidence at hand would be premature. Further work on its infective ability on a variety of hosts is under way.

The unexpected fact that the binucleate strains of *G. juniperi-virginianae* isolated here can infect both the rosaceous hosts and cedar requires further explanation. Olive (**33**) feels that infectivity in *G. clavipes* may be based upon nuclear condition of the thallus and suggests that binucleate basidiospores might be capable of producing an infection upon the rosaceous host which would give rise directly to aecia and possibly also infect the gymnosperm host. The infection results reported here indicate that Olive's prediction may be sound. In the closely related *G. bermudianum* both the aecial and telial stages occur on cedar and no rosaceous host is known. Thurston (**40**) has shown that, in these mixed infections of aecia and telia upon the same cedar gall, the fructifications arise respectively from mono- and dikaryotic mycelia.

[3] After this article had been prepared for publication my attention was called to the extensive experiments on infection of rosaceous hosts by various species of *Gymnosporangium* in the Arnold Arboretum reported in the following publications:

Crowell, I. H. 1934. The hosts, life history and control of the cedar apple rust fungus *Gymnosporangium juniperi-virginianae* Schw. Jour. Arnold Arb. **15**: 163–232.

——. 1935. The hosts, life history and control of *Gymnosporangium clavipes* C. & P. Jour. Arnold Arb. **16**: 367–410.

MacLachlan, J. D. 1935. The hosts of *Gymnosporangium globosum* Farl. Jour. Arnold Arb. **16**: 88–142.

These investigations indicate the range of infectivity shown by these fungi and suggest the possibility that ecological conditions may influence the ability of species of *Gymnosporangium* to infect specific hosts.

On this basis the uninucleate isolates obtained in this study should be capable of infecting cedar since it is known that both uninucleate and binucleate mycelia can develop within the same axenic culture. However, as indicated in TABLE III, this did not happen. It is more probable that the ability of a strain of *Gymnosporangium* to infect either or both of the hosts is determined genetically, rather than by the particular nuclear number of the infecting cell. Ultimately the factors for infectivity in this species may be shown to segregate in the same way as the factors for compatibility, which, of course, would provide a ready explanation for our experimental results.

The characteristic alterations in the infected-tissue cultures which preceded the emergence of the seven isolates may be of considerable importance in interpreting the ability of these fungi to grow saprophytically. In the period immediately before the rust mycelium grew out of the tissue cultures, callus growth declined, the amount of chlorophyll in the host cells decreased and the texture of the cultures became very soft and flaccid. As the rust mycelium became independent the infected host cells were severely damaged or lysed. This is atypical behavior for a rust fungus where the very survival of the parasite depends upon the presence of functional host cells. It is almost axiomatic that when a host cell succumbs the rust mycelium infecting it also dies. Thus cell damage following rust infection is usually indirect, in contrast to the behavior shown by many facultative parasites which kill their hosts by the liberation of toxic substances. The appearance, therefore, of a pathogenic phase during the transformation of the rust from an obligatory parasitic condition to a saprobic state was completely unexpected. Furthermore, this pathogenic phase was apparently transient, since upon reinoculation of the hosts with the isolated mycelium grown in axenic culture these seven strains again behaved as strict obligate parasites and did not produce a destructive pathogenesis.

In attempting to explain this situation, the similarity in appearance and behavior of the callus cultures at the time when the rust fungus was emerging and of those callus cultures which became habituated in Gautheret's sense should be recalled. Morphologically the appearance of the cultures under these two conditions was identical but in one case the rust fungus emerged and became independent, whereas, in the other the habituated cultures slowly declined in vigor and finally died. Whether this similarity is more than coincidental cannot be answered with the present evidence but it is tempting to speculate that the habituation of the host cells may be the cause of this apparently destructive pathogenesis rather than the activity of the rust mycelium itself. Such

an explanation would account for the lack of pathogenicity when the isolated saprobic strains are reinoculated into their respective hosts. It could be postulated that when a host-tissue culture becomes habituated the condition of anergy would allow any potentially saprophytic tendencies in the rust population to be expressed. Habituation would then operate as the screening mechanism which selects saprophytic mutants from a predominantly obligate population. The answer to this problem must await the results of studies currently under way on the retention of saprophytic tendencies in these isolates after repassage through their hosts.

The most puzzling question concerning these saprobic strains is the fact that they appeared so infrequently and that their emergence was completely unpredictable. If the transformation from an obligate to a saprobic condition involved the entire population of rust cells in a given callus culture one should be justified in asking why more independent strains were not secured in this way, since many dual-membered cultures were maintained for long periods under more or less identical conditions. It appears probable that only an occasional rust cell was capable of independent existence and that the technique used was sometimes successful in screening these potentially saprobic clones from the entire rust population in these cultures. Conversely, certain rust cells might be capable of adaptive behavior and could, in the absence of competition under cultural conditions, serve as the progenitors of strains which are capable of independent growth without the loss of their parasitic ability. Further data on this question will be presented in a subsequent publication concerning the isolation in axenic culture of other rust species.

SUMMARY

Through the use of a tissue-culture technique, systemically infected dual-membered callus cultures have been established from the telial galls of *Gymnosporangium juniperi-virginianae* produced on *Juniperus*, and maintained for several years by periodic transfer. Seven strains of this rust have been isolated from these tissue cultures and grown in axenic culture in the complete absence of the host cells. They are able to survive continuous transfer on various synthetic media. All of these strains after axenic culture proved able to reinfect their alternate rosaceous hosts in tissue culture and under field conditions, and four of them, which were presumably dikaryotic, were also capable of infecting *Juniperus*. Infection studies on various hosts indicate that one strain is

atypical in attacking both *Crataegus* and *Pyrus* and showing the characteristics of *G. globosum* when parasitizing *Crataegus*.

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NOTES AND BRIEF ARTICLES

Two little-known Ascomycetes attacking Filicales[1]

While studying parasitic fungi from the northern part of the American Cordillera I recently came upon specimens of two ascomycetes on ferns that seem largely to have escaped observation since they were described. Later, a search was made in the phanerogamic herbarium of this Division (DAO), whence the remaining records stem. The specimens cited are now in DAOM.

Trabutiella filicina (Sacc. & Scalia) Th. & Syd., Ann. Mycol. **13**: 359. 1904.

Phyllachora filicina Sacc. & Scalia, Harriman Alaska Exped. **5**: 27. 1904.

Forms small, circular tar-spots on fronds of *Polystichum lonchitis* (L.) Roth. Asci 64.0–70.0 × 9.5–15.0 μ. Spores 8, more or less biseriate, 12.0–17.5 × 3.0–5.0 μ, hyaline, continuous, subcylindrical to ellipsoid. Paraphyses absent. Microconidia present in some over-mature specimens.

Specimens examined (not all in good fruit): Rocky Creek, Kenai Peninsula, Alaska, *Calder 5189;* Mt. Thornhill, near Terrace, B. C., *Calder, Savile & Ferguson 13320;* Pine Pass, B. C., *Calder, Savile & Ferguson 12615;* Copper Knob, Mt. Revelstoke Nat. Park, B. C., *Calder & Savile 10908;* Mt. Arrowsmith, Vancouver I., B. C., *Calder, Parmelee & Taylor 19433;* Hamilton Falls, Yoho Nat. Park, B. C., *McCalla 4570;* near Cheam, B. C., 15 Aug. 1901, collector unknown; above Bertha L., Waterton Lakes Nat. Park, Alta., *Moss 480.*

This species seems to have been known previously only from the type collection taken at Unalaska. The season of maturity seems to vary considerably with location, altitude and probably season, but mature asci are most likely to be found in late June or July. In 1953 (an early season) the specimen taken at Copper Knob, Mt. Revelstoke Nat. Park, at 6000 ft, on 23 July was overmature. The fungus may be nearly co-extensive with its host in the Cordillera, but it has not been found on specimens from eastern North America. A search in herbaria with abundant Eurasian material seems to be advisable.

[1] Contribution No. 1752 from the Botany and Plant Pathology Division, Science Service, Canada Department of Agriculture, Ottawa, Ontario.

Mycosphaerella botrychii (Rostr.) Savile comb. nov.
Sphaerella botrychii Rostr. Bot. Tidsskr. **22**: 265. 1899.

The fronds affected by this fungus rarely remain flat and with substantial uninvaded areas. Only under these conditions does a leaf blotch result that strongly suggests a fungal infection. This was the symptom expression seen in a phanerogamic collection of *Botrychium multifidum* var. *intermedium* from Cold Bay, Alaska, that led me to search for other specimens of the fungus. Usually the old, infected fronds are strongly curled and uniformly black, and easily pass for normally shrivelled fronds of the previous year. Only under a lens can they be seen to be covered by the crowded, carbonous perithecia. A composite description, based on the available specimens in good fruit, follows:

Asci 30.0–42.0 × 5.0–7.5 μ. Spores 8, more or less biseriate, 11.5–17.0 × 1.6–2.0(–2.2) μ, hyaline, centrally 1-septate, fusoid. Paraphyses absent.

A search of *Botrychium* spp. in DAO has yielded the following records of the fungus. On *Botrychium multifidum* (Gmel.) Rupr. var. *multifidum:* Sibley Peninsula, Thunder Bay Dist., Ont., *Garton 1277;* Old Factory, James Bay, Que., *Lepage 32,200;* Eastmain, James Bay, Que., *Lepage 33,448;* Nominique, Labelle Co., Que., *Roy 2683;* Goynish R., Gulf of St. Lawrence, Que., *Marie-Victorin & Rolland-Germain 18109;* Natashquan, *ditto 18107, 28,201;* Norbotten, Sweden, Sept. 1902, *Frisendahl.* On *B. multifidum* var. *intermedium* (Eaton) Farw.: Frosty Creek, Cold Bay, Alaska Peninsula, Alaska, *Schofield 2496;* Summit L., between New Denver and Nakusp, B. C., *Calder & Savile 10023, 10975;* South Reserve, Sask., *Rowe 181;* Port Alexander, Renfrew Co., Ont., *Cody et al. 9415;* Valcartier, Que., Aug. 1925, *Marie-Victorin.* On *B. dissectum* Spreng. var. *obliquum* (Muhl.) Clute: Ironside, Gatineau Co., Que., *Marie-Victorin 17,428.*

In response to an enquiry, Prof. J. A. Nannfeldt kindly supplied the following information (*in litt.* 12 June 1957). There were no specimens of *Mycosphaerella botrychii* in the mycological herbarium at Uppsala, but a search in the phanerogamic herbarium yielded no less than 19 specimens from Finland and the northern half of Sweden on *Botrychium multifidum.* Among the latter are several from the neighborhood of Piteå, the type locality, which Saccardo (Sylloge Fungorum **16**: 476. 1902) erroneously gave as Denmark. It thus appears that the fungus is widespread in Europe, as in North America, in the northern part of the range of *B. multifidum* but scarce or absent in the southern part. This seems to be the first report of the fungus in North America.

—D. B. O. SAVILE, Botany and Plant Pathology Division, Science Service, Dept. of Agriculture, Ottawa, Ontario.

NOMENCLATURAL NOTES. III. CHLOROCIBORIA VS. PICEOMPHALE

Ramamurthi, Korf and Batra [1] published a validating Latin diagnosis for the genus *Chlorociboria* Seaver, and treated the six species of the genus known from North America and Europe. More recently Korf [2] discussed and illustrated nine species of the genus from Japan, six of which appear to be undescribed taxa.

Some months before the paper validating *Chlorociboria* appeared, however, the young Czechoslovakian discosystematist Mirko Svrček [3] erected a monotypic new genus, *Piceomphale*, based upon *Peziza bulgarioides* Rab., which he (correctly) assigned to the Ciborioideae (= Sclerotiniaceae sensu meo). This same species was treated by Ramamurthi, Korf and Batra as a species of *Chlorociboria*.

Under the current International Code of Nomenclature (Paris), the generic name *Chlorociboria* Seaver *in* Ramam., K. & B. must now be abandoned. It was nomenclaturally superfluous when published, since it included the type of a name (*Piceomphale*) which ought to have been adopted under the Code; it is thus illegitimate (Art. 64) and must be rejected (Art. 63).

The valid name for the generic concept treated by Ramamurthi, Korf and Batra is now *Piceomphale* Svrček, the generic diagnosis of which would need only slight amplification in order to include the aeruginous species. Svrček did not indicate the gender of his generic name, but since the classical gender of *Omphale* in both Greek and Latin is feminine, it would seem best to treat the name as feminine.[4]

[1] Ramamurthi, C. S., R. P. Korf & L. R. Batra. 1958. A revision of the North American species of *Chlorociboria* (Sclerotiniaceae). Mycologia **49**: 854–863. (1957.)

[2] Korf, R. P. 1958. Japanese discomycete notes I–VIII. Science Rept. Yokohama Nat. Univ. II **7**: 7–35.

[3] Svrček, M. 1957. *Piceomphale bulgarioides* (Rabenh. in Kalchbr.) Svrček comb. n. a poznámky k problematice diskomycetu *Ombrophila strobilina* v pojetí Rehmově. Taxonomical and nomenclatorical notes on discomycete *Ombrophila strobilina* sensu Rehm. Česká mykologie **11**: 235–240.

[4] The name *Piceomphale* and the nomenclatural problems have been discussed in correspondence with Professor D. P. Rogers, University of Illinois, whose helpful comments I wish to acknowledge. The name, superficially, seems to be derived from the generic name of the host of the type species, *Picea*, or alternatively *piceus* (L., *-a, -um*), "pitch black," plus *Omphale* (L., *-es*, f., taken from Greek), "a

My studies of Japanese discomycetes have led me to a critical reinvestigation of the type species of *Chlorosplenium*, *C. chlora* (Schw.) Curtis *in* Sprague. As I have shown elsewhere,[5] this species does not differ in truly fundamental characters from the species which have been segregated in *Chlorociboria* and *Piceomphale*. In consequence, I am abandoning both of these names, and reverting to *Chlorosplenium* for all of these species. Because of this taxonomic decision, it is unnecessary to propose a transfer of the species treated in *Chlorociboria* to *Piceomphale*.

Ramamurthi, Korf and Batra tentatively suggested that it might be advisable to segregate into separate genera the aeruginous species of *Chlorociboria* from the olivaceous to black species. It is a peculiarity of our current Code that there seems to be no provision to reinstate *Chlorociboria* for the aeruginous species if a division of *Chlorosplenium* or of *Piceomphale* should be desired by future workers. Although a "reinstated" genus *Chlorociboria* would be neither a later homonym (if also based on *Helotium aeruginosum*) nor a synonym (since it would not be *another* name), it would still be the same name, and hence "must be rejected," and a new name would need to be chosen (Art. 72). On the other hand, if *Chlorociboria* had been published before *Piceomphale*, or if *P. bulgarioides* had been omitted when *Chlorociboria* was validated, both names would still be available for a future division of the genus.—RICHARD P. KORF, Department of Plant Pathology, Cornell University.

MYXOMYCETES FROM IRAQ

The mycological flora of the Republic of Iraq has not been explored except in the papers published on Iraqi soil fungi by Tolba et al. (1) and Al-Doory et al. (2) which dealt with the central area of the country only. Thus far there have been no publication on the Myxomycetes.

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queen of Lydia, whom Hercules served." It seems far more likely that the root intended was *omphalos* (L., *-i*, m., taken from Greek), "the navel." I prefer to accept the feminine root as written, rather than changing the spelling and treating the generic name as an orthographic error. Rogers has also pointed out to me that S. F. Gray treated the agaric genus *Micromphale* Gray, Nat. Arr. Br. Pl. 1: 621. 1821, as neuter, in the false belief that names ending in *-e* are third-declension neuters, whereas *-e* is a normal termination for Greek first-declension feminines. Judging by the English name, "dimple-stool," and the description, "*tip* [of cap] umbilicated," Gray also apparently had *omphalos* rather than *Omphale* in mind.

[5] Korf, R. P. 1959. Japanese discomycete notes IX–XVI. Bull. Nat. Sci. Museum (Tokyo) (in press).

This report includes three species of Myxomycetes which were collected in the first two weeks of March, 1958, within a radius of about ½ mile of the suburban area of Diwania city (Ghammas, Diwania Liwaa), which is located in the southern part of Iraq and has an altitude of a few feet above sea-level.

The weather in this area during the months of December through March is cold, rainy and humid. Also, dust storms occur frequently.

I wish to thank Dr. C. J. Alexopoulos of the State University of Iowa for his assistance in identifying the specimens.

ORDER PHYSARALES, FAMILY PHYSARACEAE

PHYSARUM CINEREUM (Batsch) Pers.

Very large number of sporangia scattered on dead leaves.

BADHAMIA GRACILIS (Macbr.) Macbr.

Many sporangia scattered on decaying logs.

FAMILY DIDYMIACEAE

DIDYMIUM IRIDIS (Ditmar) Fries

Few sporangia scattered on the bark of a dead tree.—YOUSEF AL-DOORY, Department of Botany, University of Baghdad, Baghdad, Republic of Iraq (present address, University of Oklahoma, Norman).

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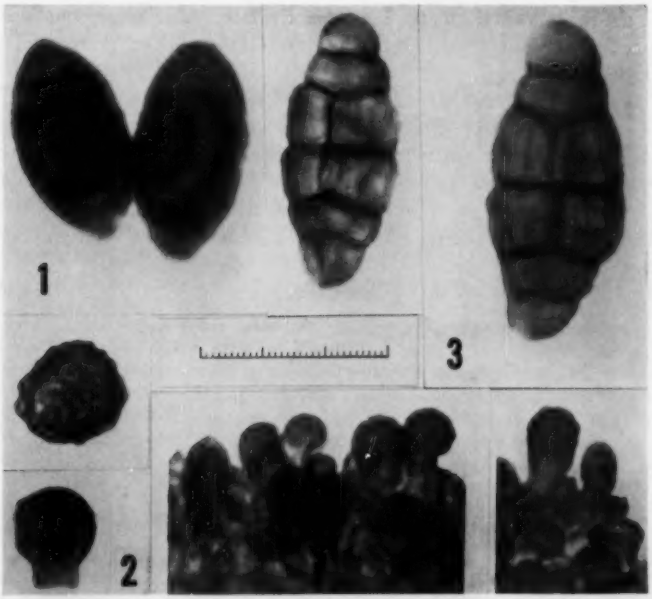
SCHELEOBRACHEA HUGHES [1]

When Spegazzini transferred to the University of La Plata he took with him part of his collection of *Sporidesmium echinulatum* Speg., "ad chartam putrem stercoratum in locis umbrosis," and left the remainder with Saccardo, thus establishing authentic material at both Herbs. LPS and PAD. However, the material deposited in LPS bears on its packet several sketches of conidia which strongly resemble

[1] A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Harvard University.

Sp. bakeri var. *bakeri*, though no spores of this type are discernible on either collection.

Hughes (2) has designated the PAD isotype as the type material of his genus. (In synonymy with *Scheleobrachea echinulata* he lists *Sp. bakeri* var. *bakeri*, subsequently (3) considered a synonym of *Piricauda chartarum*.) This material bears on it three distinct fungi: 1) dictyospores, diagnosed below, that are dematiaceous, glabrous, fuscous,



Elements present in the type-material of *Sporidesmium echinulatum* Speg. FIG. 1. Unidentified phaeospores. FIG. 2. *Scheleobrachea echinulata*, lectotype: right, portions of sporodochia; left, two disrupted conidia. FIG. 3. *Piricauda scorobylos*, type, two typical conidia. Scale in micra.

paucicellular, ellipsoid, and scattered diffusely over the substrate; 2) asperate conidia that are borne monacrogenously on short, single-celled conidiophores that expand into, and are terminated by, globose, fuscous, papillose to tuberculate amerospores, 7.5–14 μ diam., produced in punctate sporodochia (FIG. 2). (It is probable that this fungus is an *Epicoccum* and that the conidia are generally in the state of maturation illustrated by Ellis (1) in his *Fig. 2d.*); 3) laevigate, deep fuscous, oval,

discharged phaeospores, 17–21 × 30–31.5 μ, that each contain a large guttula (FIG. 1).

Though Hughes characterizes the conidia of *Scheleobrachea* as being "amerosporia vel phragmosporia vel dictyosporia," "pallide brunnea vel brunnea, ovalia, obovalia, fusoidea vel ellipsoidea," the fact that he says they also are "solitaria in apice denticularum oriunda" and borne on "conidiophora brevissima, numerosa, interdum congesta cylindracea, forma denticularum irregulariter in hyphis producta" leads one to infer that the sporodochial fungus, Form-family Tuberculariaceae, most nearly approximates the type description, and this is here designated the *lectotype*.

Hughes transfers four other fungi that he considers to be congeneric: *Sch. maydica* (Sacc.), *Sch. philippina* (Sacc.), *Sch. sacchari* (Speg.), and *Sch. quadrata* (Atk.). The last two named species and their synonyms are considered to be elements of *Piricauda* (3) and are treated under *P. sarkara* and *P. quadrata* respectively, while *Sch. maydica* is discussed under *P. chartarum*. *Sch. philippina* has not been seen.

Piricauda scorobylos sp. nov. Fig. 3

Conidia in mycelio sessilia, mellea, ellipsoidea aut leviter clavata, laevigata, vulgo octocellularia, interdum decemcellularia, ad septa nonnihil constricta, utrinque e duabus cellulis simplicibus constans, in medio e duabus seriebus cellularum dictyodeis, (vulgo quadripartitis, interdum sexpartitis), (13.5–)18.5–21.0 × (34.5–)47.5 (–52.5) μ.

Ad chartam putrem stercoratum in locis umbrosis prope Conegliano, Italia, cum *Scheleobrachea echinulata* (Speg.) Hughes, TYPUS, in Herb. PAD, (preparatio microscopica RTM I:260, isotypus).

(Etm. σκοροβυλος —a beetle, in fancied reference to the cellulation of the conidia.)

This work was done under the guidance of Dr. I. Mackenzie Lamb, Director of the Farlow Herbarium, to whom acknowledgments are also made for his kindness in the preparation of the Latin diagnosis.—R. T. MOORE,[2] Dept. of Biology, Harvard University, Cambridge, Mass.

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REVIEWS

PHYSIOLOGY OF FUNGI, by Vincent W. Cochrane. xiii + 524 pp., *illus.* John Wiley & Sons, Inc., New York, 1958. Price, $9.75.

This book reflects the special interest of its author in metabolism as well as his varied experience as an industrial microbiologist, plant pathologist, and teacher of biology. This book also serves as evidence that during the past fifteen to twenty years, Vincent Cochrane has been industriously reading. Each chapter is concluded with a bibliography and together the bibliographies contain the impressive total of 4480 references. This reviewer found the average number of citations per page to be twenty-one, as determined by counting the citations on ten randomly chosen pages. One reading of the text leaves no doubt that Cochrane has read all of the papers cited and many more. Early and recent publications through 1956 are listed and references to papers published in 1957 bear witness to the author's effort to have the book as up-to-date as possible. Only rarely can the specialist reading this book find that an important paper in his field has been omitted. Seldom is the reader forced to consult the paper cited in order to understand the sentence in which the reference is made. One example of this rare occurrence is found on page 251 where we read, ". . . The distribution of proteolytic enzymes between mycelium and culture fluid is conditioned by age (185) and the medium (208). This may explain some apparent disagreements (130, 142)." Only by reading papers 130 and 142 can one learn what the apparent disagreements are. The citation of papers is not, however, indiscriminate. The author has read critically as well as extensively and he does not hesitate to pass judgment on the merits of many of the papers cited.

The purpose of the book is, as stated in the preface, "to summarize and organize our knowledge of the physiology of the fungi, indicating whenever possible some of the problems that lie immediately ahead." The author has succeeded admirably in accomplishing this purpose, particularly in regard to the nutritional and metabolic studies of the fungi to which almost two-thirds of the book is devoted. Although the treatment accorded fungous metabolism is one of the major assets of this book, the chapters dealing with growth, composition of fungal cells, and the actions of physical and chemical agents on fungi are also valuable.

Descriptive studies of such fungous activities as development of fruiting bodies and discharge of spores have been excluded on the grounds that the physiological and biochemical determinants of these activities are largely unknown at this time.

Dr. Cochrane's style of writing is clear and effective. There are twenty-three tables of information, compiled by the author, and seventy-four figures. The figures, most of which are graphs, have been well chosen to illustrate physiological effects discussed in the text.—ALMA W. BARKSDALE.

MYCOLOGIE MÉDICALE. Communications et rapports présentés aux Journées de Mycologie Médicale (14–15 décembre, 1956), organisées par l'Institut Pasteur et le Société Française de Mycologie Médicale. 344 pp., *203 figs. & tab., 6 pl. col.* L'Expansion Scientifique Française, Paris, 1958. Price, 3000 fr.

That medical mycology has flourished in France can be attested to by anyone who has tried to describe a "new" dermatophyte, understand the medical concepts "sporotrichosis" or "aspergillosis," or trace the origin of such terms as "fuseaux," "aleuries," "ectothrix microides" or even 'Sabouraud's conservation medium."

That this interest still flourishes is amply demonstrated by the present volume, as well as by the two-day conference that gave rise to it. The conference, sponsored by the Pasteur Institute and held in Paris in December, 1956, marks the official inauguration of the French Society of Medical Mycology. Besides a stimulating "Preface" by the Institute's Director, J. Trefouël, and a sobering introduction by the sponsoring Society's President, E. Rivalier, the volume contains 35 additional contributions by 74 authors—Drouhet, Mariat, Segretain and Vieu being among the best known and most productive of the group.

The long interval between the date of the conference and this publication probably explains why all but four of these papers have appeared elsewhere (Semaine Hôpital, vol. 33, for 1957); yet their handy collocation in this volume makes its purchase a wise investment for the well-chosen comprehensive medico-mycological bookshelf.

The papers are arranged under six sections: I, Mycoses of the respiratory tract; II, Mycoses of the central nervous system; III, Mycoses due to *Candida;* IV, Dermatomycoses; V, Exotic mycoses, and VI, Antifungals and the therapy of the mycoses.

The first section deals exclusively with the broncho-pulmonary aspergilloses in five independent papers. Of particular interest to

American physicians and diagnostic laboratories are the papers describing various aspects of a peculiar type of bronchiectatic aspergilloma, a remarkable, fairly easy-to-cure condition, of which only a few unpublished examples appear to have been recognized in this country.

In the second section, in addition to a few isolated case reports of unusual types of infection by the ubiquitous *Cryptococcus neoformans* or the less common *Cladosporium trichoides*, there is an excellently didactic review by F. Mariat on the mycological diagnosis of the principal mycoses of the central nervous system. Here are presented by means of excellent comparative tables, lucid text and illustrations, (1) a review of the various fungi reported to invade the C.N.S. and (2) a summary of the various cases in the literature showing cerebral involvement by *Rhizopus, Cladosporium* or *Nocardia*. This paper alone justifies the entire section.

Under the topic "Mycoses by Candidas," 13 papers are included: well-documented discussions of *Candida albicans* infections in the newborn (Debré and others); pulmonary moniliasis in premature infants (Larroche); vaginal moniliasis (Grasset *et al.*); and two excellent papers by E. Drouhet on the biology of *Candida* infections: one in collaboration with M. Vieu on their laboratory diagnosis, and a second one on the pathology and etiology of 175 cases of moniliasis. Worthy of note also are the investigation (Bernard *et al.*) of the etiologic factors favoring superinfections by *C. albicans* in malignant blood diseases and the review (P. de Graciansky) of the cutaneous and mucosal involvement by yeasts during the course of antibiotic therapy. Papers on therapy of, and allergy due to, these infections and on the yeast-like bronchial flora in sanatorium patients complete this section. Of particular interest in this section is a paper by P. de Graciansky and collaborators reporting a study of the influence on experimental *C. albicans* infections of nutritional deficiencies in the white rat—a gratifying departure from the purely descriptive and didactic.

Section IV reports in separate papers on the present-day dermatophytic flora of Paris, Lyons, Marseilles, the north of France and the French Cameroons. The influx of North African laborers to the Paris area has increased the incidence of *T. schoenleini* infections, but this fungus and also *M. audouini* are gradually diminishing in the native French population.

Section V, in three well-illustrated papers, discusses mycetoma, both actinomycotic and maduromycotic, from the histopathological and parasitological points of view. In two other papers, the giant forms of *Histoplasma* and some aspects of the biology and mycology of histo-

plasmosis are discussed. Finally, one paper each on chromoblastomycosis in Madagascar and on rhinosporidiosis complete this section, of particular interest to those dealing with mycoses in tropical areas.

Section VI contains two papers, one by Drouhet, who, after outlining the difficulties in controlling systemic fungi *in vivo*, proceeds to list the characteristics and activity of several physical, chemical, and antibiotic antifungal agents. Finally, each disease is discussed individually, outlining the most promising therapy currently available. As usual with Dr. Drouhet's papers, the synoptic presentation clarifies the text; nystatin is highly praised. A final paper by M. Rimbaud and others presents a study of the action of dichloroxyquinaldine on experimental intestinal moniliasis in dogs. The latter could only be established in dogs on a low-protein, high-carbohydrate diet. Here again a departure from the purely descriptive is welcome.

The volume points to an activity in the study and dissemination of knowledge of the human mycoses in France and its colonies from which even the best-informed mycologists and physicians in our country can profit. The scarcity of papers on experimental work suggests the need for a second volume dealing with experimental studies.

The quality of the printing, including typography, half-tones, and color illustrations, is superb; summaries in English, Spanish and German extend the volume's usefulness. The size of the pages and their number make a hard-cover binding, now absent, almost essential.—MARGARITA SILVA.

THE CHEMISTRY AND BIOLOGY OF YEASTS, edited by A. H. Cook. xii + 763 pp. Academic Press, New York, 1958. Price, $22.00.

Good organization by the editor and a high level of writing by the contributors have created an excellent book. Its wide scope, uniform excellence in quality of information and readability make the book stand alone in the field of zymology. An immense amount of factual material is given in each area covered, and the areas are manifold. Dr. Lodder and her colleagues at CBS in Delft write authoritatively on the classification of yeasts; Winge and Roberts cover in a dignified manner the relatively new field of yeast genetics and the growing pains it currently suffers, as well as the older and even more confused interpretations of cytology. Ecology (Lund), food spoilage (Ingram), and pathenogenicity (Ainsworth) describe some interesting activities of these active fungi. Chemical composition of yeasts (Eddy), yeast growth (Morris), fer-

mentation and respiration (Nord and Weiss), synthesis and degradation of cellular carbohydrates by yeasts (Trevelyan), and nitrogen metabolism (Harris) are all firmly based on a large amount of chemical data. As only one chapter is devoted to the technology of yeasts (Pyke), it may be inferred that the coverage of the book is intended to be wide and general. Pyke's contribution is brief, modern, and useful. Jansen writes on flocculation of yeast, a many-faceted problem with which European brewers are commonly concerned.

This much-needed book is so very well done that zymologists everywhere might wish it could be repeated every 5 or 6 years to keep interested persons informed of advances in this active field. Zymology is too small to warrant a yearly book-length review, and it is inadequately covered in such reviews of the fungi. Indeed, an excellent service would be rendered by Dr. Cook and his associates at the Brewing Industry Research Foundation if they could accept responsibility for a continuing series of similar books.—LYNFERD J. WICKERHAM.

LES URÉDINÉES. TOME III. GENRE UROMYCES, by A. L. Guyot. Encyclopédie Mycologique. 647 pp., 143 figs. 1957. LES ROUILLES DES LÉGUMINEUSES FOURRAGÈRES ET SPONTANÉES. 647 pp., 143 figs. 1957. Paul Lechevalier, Paris. Price, 8500 francs (about $18.00) each. Paper bound.

The French are supposed to be a very logical people. It is logical for Lechevalier to assume that more sales would result if the same book were to be published under two titles and the fact not mentioned in the advertising. So, violá. It is noted on the "fly title" of each volume that the work was issued under another title but this discovery costs about 18 American dollars if one happens to buy both volumes. This may make the deal legal but it will make me very suspicious of future listings by Paul Lechevalier.

This volume represents (or these volumes represent) a monograph, primarily of the European species of *Uromyces* that parasitize legumes, but the extra-European species are listed. As in the previous volumes, Guyot utilizes sections, in this case sections: A, verrucosi-*Uromyces* with verrucose teliospores; B, reticulati-*Uromyces* with reticulate teliospores; C, crassi-*Uromyces* with teliospores having smooth, apically thickened teliospores; D, species of *Uromyces* not assignable to sections A, B, or C; and E, consisting of species recently transferred to other genera. Section A, comprising 111 species in 8 subsections, is the

largest; Section B has 21 species; Section C has 30 species; and Section D has 21 species; or a total of 183 species.

Prof. Guyot implies, by his treatment, that he does not really believe that there are so many species. For example, Section A, subsection *a*, entitled "sous-section correspondant au type **Uromyces anthyllidis** (Grev.) Schroet." is composed of 42 "species" most of which are illustrated and described although few of them are morphologically distinct. *Uromyces trigonellae* Pass. is treated as a species but the associated figures use *Uromyces anthyllidis* (Grev.) Schroet. *s. l.* in the legends. All of this means that, while there is a great amount of information in the book, there is also a great amount of useless duplication. Prof. Guyot would have done a far greater service had he utilized his extensive knowledge of the legume rusts to set out a clearly recognizable system of species and consigned to synonymy those so-called species based on host or geography. Revision of this kind is long-overdue but it is not provided by this voluminous and expensive book.—GEORGE B. CUMMINS.

MYCOLOGICAL PAPERS—The Commonwealth Mycological Institute has recently published, as no. 70 of the series of *Mycological Papers*, "*Clasterosporium* and some allied Dematiaceae-Phragmosporae. I," by B. M. Ellis (89 pp., 63 figs. $4.50), and has in press no. 71, "Studies on *Cercospora* and allied genera. I," by F. C. Deighton, and no. 72, a continuation (II) of no. 70, also by Ellis. These are the most recent numbers of a series now going into its twenty-eighth year, which has included many important and continually useful contributions to mycophytopathology, mycogeography, and systematic mycology. All of the back numbers are still available, and a continuing subscription (which entails a rebate of ⅓ from published prices) can be considered a necessity for an institutional mycological library.

Since even well-stocked libraries may lack some of the other equally useful publications of the C.M.I., a note may be inserted here as a reminder. The *Index of Fungi*, listing all new genera, species, and varieties of fungi, beginning in 1940, is issued twice a year. It is backed up by C.M.I. reprints of Petrak's similar lists of new fungi, originally printed in the Bot. Jahresber.; eight of these carry the indexing back to the beginning of 1920; cumulative indexes are available. Together the *Index of Fungi* and Petrak's lists supply names and references for all fungi published since Saccardo's *Sylloge*. An annual *Bibliography of Systematic Mycology* has been issued for 1947 and all succeeding years.

And microfilm copies of certain mycological classics are available. The *Review of Applied Mycology,* universally relied on by phytopathologists and industrial mycologists, has published summaries of the literature in its field beginning with 1922. The *Review of Medical and Veterinary Mycology,* begun in 1942, offers the same service in another phase of mycology. The *Distribution Maps of Plant Diseases,* of which 365 have appeared, carry supporting text as well as complete mapping. A *Catalogue of the Culture Collection of the C.M.I.* is kept up-to-date by semiannual supplements and periodical reissue. A new series of *Phytopathological Papers,* begun in 1956, now includes three numbers, with a fourth in preparation. Of the numerous books and other publications treating various phases of mycology and phytopathology the most generally used is the Ainsworth & Bisby *Dictionary of the Fungi;* other useful and more special works have appeared.

Lists of prices and titles for all these publications can be obtained from the Commonwealth Mycological Institute, Kew, Surrey, England. —D. P. R.

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